The use of enzymatic kinetic and dynamic kinetic resolutions in organic synthesis

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The Use of Enzymatic *Kinetic* and *Dynamic Kinetic* Resolutions In Organic Synthesis

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Abstract

Enzymes have found many applications in synthetic chemistry both in industry and academia. They have been specifically used to achieve kinetic resolutions within the field of asymmetric synthesis and of late have been employed in parallel with racemising agents to facilitate dynamic kinetic resolutions.

The kinetic resolution of Evans oxazolidinone has been attempted using a variety of lipases, esterases and proteases. The enzyme *Candida antartica* lipase was found to catalyse both the acylation and hydrolysis reactions of the valine derived oxazolidinone at 40 °C but facilitated no kinetic resolution of the substrate.

The kinetic resolution of pantolactone chiral auxiliary has also been attempted. In this instance successful *kinetic resolutions* have been achieved through, acylation of pantolactone acetate and the acylation, hydrolysis and transesterification of pantolactone acrylate using the lipase *Pseudomonas cepacia* lipase. For example, pantolactone can be resolved through the formation of its acetate in 99% ee using vinyl acetate as an acyl donor, 49% conversion was achieved after 8h at r.t.. *Pseudomonas cepacia* lipase has also been employed in studies towards the diastereomeric differentiation of Diels-Alder adducts of pantolactone acrylate.

The *kinetic* resolutions of α-chloro esters have also been accomplished using lipase catalysed ester hydrolysis. We have found that both *Pseudomonas cepacia* lipase and *Candida cylindracea* lipase (*Candida rugosa* lipase) will catalyse the hydrolysis reaction of a number of α-chloro esters. For example, the methyl ester of α-chloro

phenyl acetic acid has been resolved using the *Candida cylindracea* lipase in buffer:solvent mixtures (5:1) giving α-chloro phenyl acetic acid in 90% ee after 16h. Further more these *kinetic* resolutions have been combined with a racemisation protocol within studies towards the dynamic kinetic resolution of α-chloro phenyl acetic acid. To facilitate the dynamic kinetic resolution of α-chloro phenyl acetic acid, a wide array of quaternary phosphonium and ammonium chlorides have been shown to racemise a variety of both aryl and alkyl α-chloro phenyl esters but not thier corresponding acids. To date a reproducible, successful, dynamic kinetic resolution has not been achieved.

Contents

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Finally, I would like to thank my family for all of their love and support.

Abbreviations

aq.	Aqueous
tBuOMe	tertiary butyl methyl ether
DMAP	4-dimethylaminopyridine
DCM	dichloromethane
de	diastereomeric excess
DKR	*dynamic kinetic* resolution
ee	enantiomeric excess
EtOAc	ethyl acetate
eq.	equivalents
GC	gas chromatography
h	hours
HPLC	high performance liquid chromatography
IR	infra-red
MeOH	methanol
mins.	Minutes
NMR	nuclear magnetic resonance
Ph	phenyl
Prep. TLC	preparative thin layer chromatography
sat.	saturated
THF	tetrahydrofuran
TLC	thin layer chromatography
wrt	with respect to

Section 1

Enzymes in Organic Synthesis

1.1 Introduction

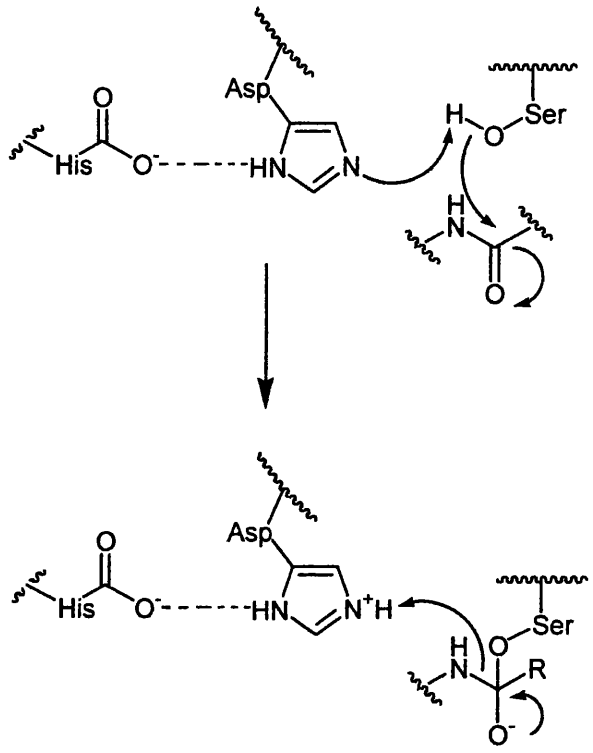
Enzymes are catalysts evolved in nature to speed up and co-ordinate the multitude of chemical reactions necessary to develop and maintain life. As catalysts-true to the definition familiar in chemistry-enzymes alter the rate in which a thermodynamic equilibrium is achieved. The increase in rate is achieved by lowering the activation energy of the overall process

In the main, enzymes are protein molecules of between 10^4-10^6 DA in molecular mass. The complex structure of an enzyme provides a unique chemical environment in which a reaction can take place. These domains are usually described as the enzyme's 'active site', areas in which specific molecules may fit and react. Substrates bind to the active site through multiple covalent interactions between themselves and the enzyme. The strength of these bonds is strongly dependent upon the distance and angle of interaction thus highly selective binding between substrate and enzyme may occur.

It is this highly selective binding which has interested and fascinated synthetic organic chemists as these properties supply a means to facilitate asymmetric synthesis, for example in the case of *kinetic* resolutions. Inherently enzymes work best under their 'natural' conditions, typically neutral aqueous solution at 20-40 °C. It is under these conditions that the active site exists at its optimum.

1.2 Mechanism

There are four understood mechanisms for proteases.[1,2] One of which is used to explain the mechanism of *Pseudomonas species* lipase which uses the amino acids aspartine, histidine and a serine to hydrolyse esters as shown in **Scheme 1**.

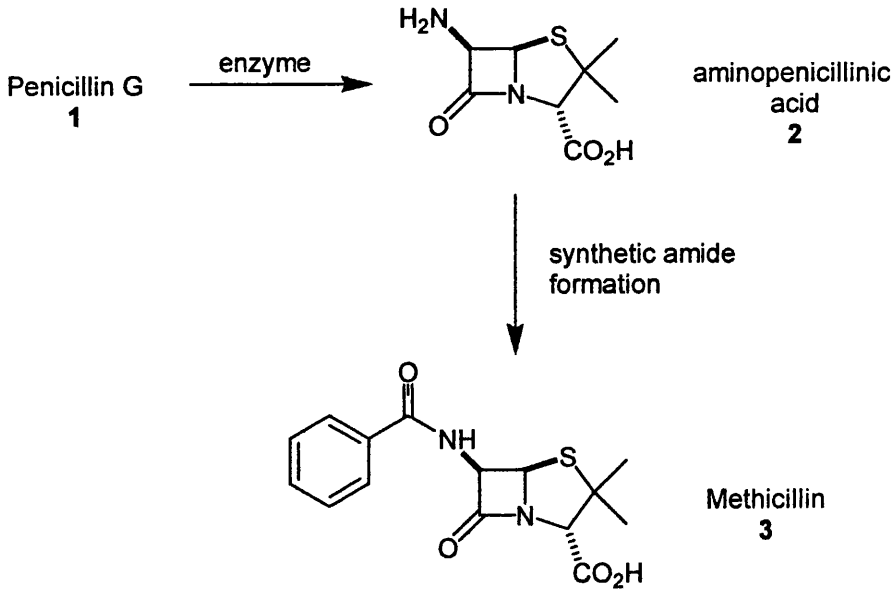


Scheme 1

The ester function is captured in the active site via the reaction of a hydroxyl functionality on the serine moiety. In the second distinct step this intermediate is de-acylated with water. In addition to water, other nucleophiles such as alcohols, amines and thiols can also react to form esters, peptides or thio esters.

1.3 Enzymatic applications

Due to their mild reaction conditions and their natural occurrence enzymes have been considered as extremely useful reagents in many industrial applications.[3] The production of Penicillin G and V by the microbial enzyme *Penicillin* acylase supplies the world with an excess of 12,000 tonnes of these penicillins per annum.[4] Further to this discovery, the hydrolysis of Penicillins has also been accomplished using the enzyme *Penicillin* amidase a technique that has rivalled alternative chemical processes in term of simplicity and cost. This has led to the production of semi synthetic penicillins such as Methicillin **3** thus, Penicillin G **1** undergoes a hydrolysis affording the intermediate aminopenicillinic acid **2**, which is subsequently N-acylated to a give Methicillin **3** as shown in **Scheme 2**.



Scheme 2

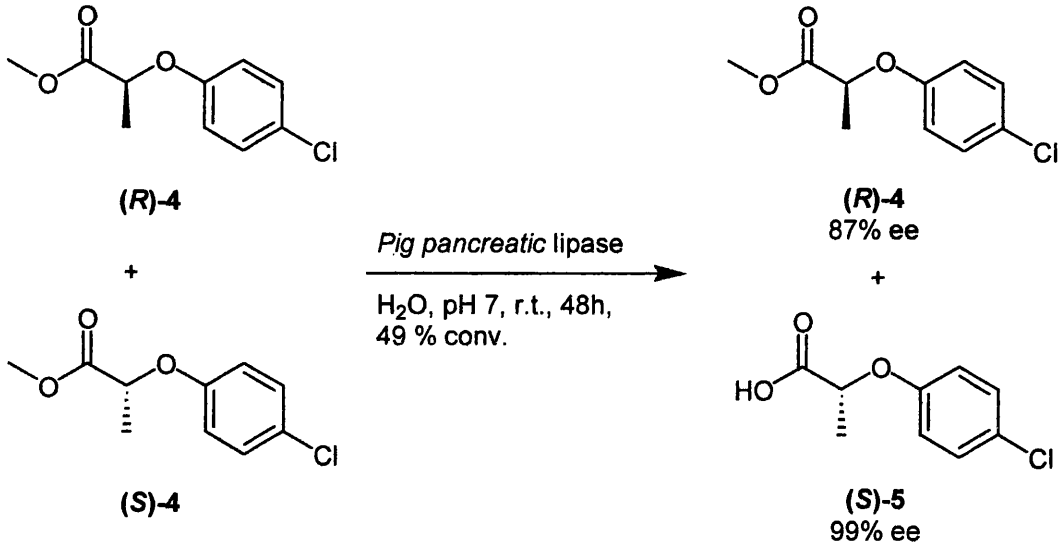
Although enzymes provide mild working conditions many organic chemists have long prejudiced them against. Enzymes are often considered to be too sensitive, too specific and not able to be used under the range of reaction conditions a chemist

would routinely employ for example, reflux and the use of organic solvents. In the last 15 years many of these prejudices have been challenged and surpassed.[5-7]

1.4 Hydrolytic enzymes and *kinetic resolutions*.

Out of the various types of enzymes available to a synthetic chemist, hydrolytic enzymes such as, proteases, esterases, lipases and acylases, have been most widely used in synthetic organic synthesis.[8-20] A lack of sensitive co-factors that would have to be recycled and a large number of readily available enzymes possessing relaxed substrate specificities to choose from are the main features that have made hydrolases so popular.[21]

Hydrolytic enzymes have typically been used to catalyse the hydrolysis of esters and amide bonds and are also able to catalyse the reverse bond forming reactions.[22] **Scheme 3** describes a typical lipase *kinetic resolution* of the racemic ester **4**. *Pig pancreatic* lipase was shown to catalyse the hydrolysis of the (*S*)-enantiomer of the ester **4** selectively, thus resolving the starting material into **(*R*)-4**-ester and **(*S*)-5**-acid[23] **Scheme 3**.



Scheme 3

If, as in this example, an enzyme can be found that achieves a very high selectivity for one enantiomer over another this makes for a very simplistic asymmetric synthetic method. The down side of enzymatic *kinetic resolutions* is that they only provide a maximum 50% yield or conversion unless a racemic product is observed.

1.5 Enzymes in organic solvents

Although the use of water as a reaction medium is cheap and environmentally friendly, it does have its limitations as a solvent. For example, many organic molecules are not in soluble in water and therefore don't react well in it. Removal of organic compounds from water is also tedious and expensive due to its high boiling point. In some instances biocatalysts are soluble in water and therefore are difficult to recycle from aqueous media.

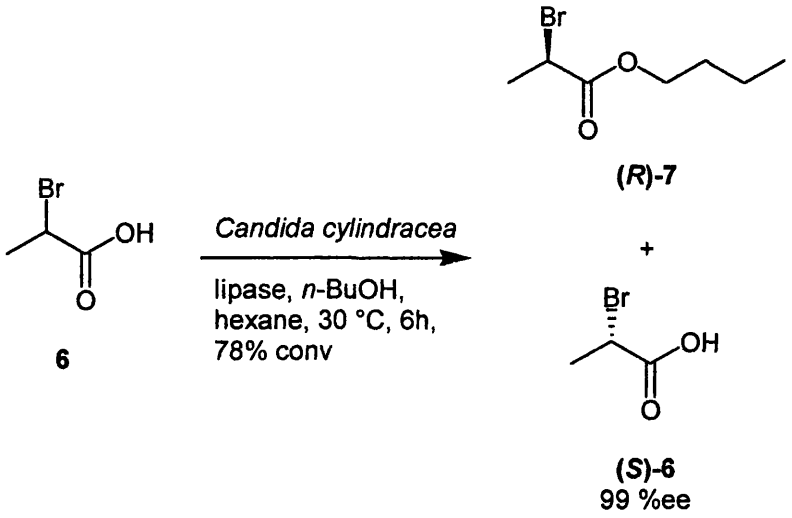
Organic chemists have overcome these shortfalls by the use of biphasic systems. Generally organic solvent of between 5-20% v/v is added to the reaction mixture to

enable dissolution of substrates and easier work up protocols. Enzyme studies in biphasic systems have showed that solvents such as octanol (2.9), ether (~2.9) or hexane (3.5) which have a log P of greater than 3.5 work best.

In the early 1980's Klibanov et al reported,[24] that although it was the commonly believed that enzymes would be denatured by the used of anhydrous reaction conditions, that this opinion is certainly not true. The opinions of the time pertaining to enzyme activity in organic media were thought to be too simplistic, since in nature many enzymes or multi enzyme complexes function in hydrophobic environments, for instance in the presence of or bound onto a membrane. On the other hand, Klibanov reported that water was absolutely required for catalytic activity of enzymes. This is because water participates directly and indirectly in non-covalent interactions that maintain the native catalytically active conformation of enzymes. Hence removal of water should drastically distort that conformation and de-activate the enzyme. However, it was proposed that it is not whether water is required but how much of is necessary to maintain catalytic activity[25].

It has been found that α-chymotripsin only needs 50 molecules of water per enzyme molecule to remain catalytically active[26]. This is much less than is needed to form a monolayer of water around the enzyme. Klibanov found that the types of solvent suitable to carry out enzymatic *kinetic resolutions* in anhydrous media follow the same trends as those used in biphasic systems. With this newfound knowledge the esterification and acylation of acids and alcohols could be achieved[27].

Klibanov reported a very successful esterification of α-bromo propionic acid **6** using butanol and the enzyme *Candida cylindracea* lipase[28,29] as shown in **Scheme 4**. The acid **6** is resolved by *Candida cylindracea* lipase in 99% ee and 78% conversion after 6h at 30 °C.

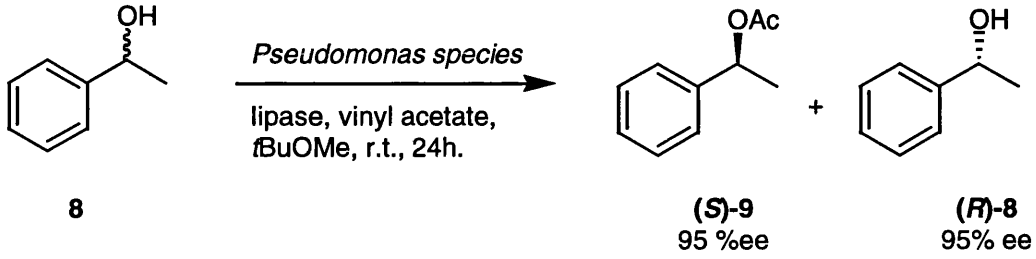


Scheme 4

This paper was certainly one of the pivotal reports that suggested that enzymes (usually immobilised) could be used successfully outside of buffered solution. Although these conditions are 'unnatural' to the enzyme they are shown to work very effectively and provide unique advantages over aqueous solution. For example, there is no need to convert the acid to an ester in order to achieve a *kinetic resolution* through hydrolysis, there-by eliminating a synthetic step.

Further to the discovery that enzymes could both hydrolyse[30] and esterify acids they were also found able to perform the acylation[18, 31-33] of a variety of alcohols and amines[34]. The acylation of alcohols has been achieved using specially designed acyl donors[35] as shown in **Scheme 5**. In this example, *Pseudomonas species* lipase was

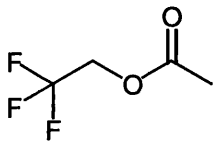
found to selectively catalyse the formation of the acetate (*S*)-**9** from racemic phenethyl alcohol. The acetate (*S*)-**9** was formed in 95% ee at 50% conversion after 24h.



8 (*S*)-**9** 95 %ee (*R*)-**8** 95% ee

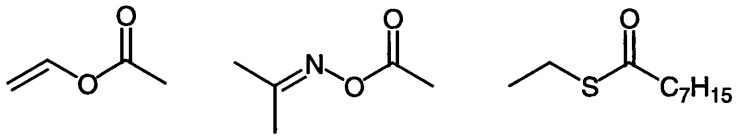
Scheme 5

The crux of these types of *kinetic* resolutions is certainly the efficiency of the acyl donor[36]. Initially activated esters **10** were used but problems arose due to the reversible nature of these acyl donors. The alcohol released upon reaction of the acyl donor was able to react with the newly formed enzyme-acyl donor complex reversing the acylation process, thus compromising conversion and potentially the overall selectivity of the acylation reaction.



10

Irreversible acyl donors such as the vinyl acetate[36-39] **11**, the oxime[40] **12** and the thio ester[41] **13** were then designed.



11 **12** **13**

Of these three acyl donors **11**, **12**, and **13**, vinyl acetate **11** is the most comprehensively used. Vinyl acetate is made irreversible because the alcohol released from the acyl donor tautomerises to produce acetaldehyde, which is unable to react further, stopping de-acylation of the acyl-enzyme intermediate occurring. Oxime ester **12** works in a similar way where as the thio ester **13** is reported to work slightly differently. In this instance Frykmann states that the thiol released upon acylation evaporates and therefore is unable to react further to reverse the acylation process.[41] Another theory is that the thiol is too poor a nucleophile to reverse the acylation process.

1.6 Enzyme Immobilisation

Nature immobilises its enzymes by compartmenting them within cell organelles or separating them using membranes. Chemists have immobilised enzymes to try and mimic nature in the laboratory. There are no hard and fast rules of when and how enzymes should be immobilised but there are three main reasons why enzymes have been immobilised prior to use.[42]

- To increase enzyme stability
- To make enzymes insoluble and thus enable them to be recycled by filtration.

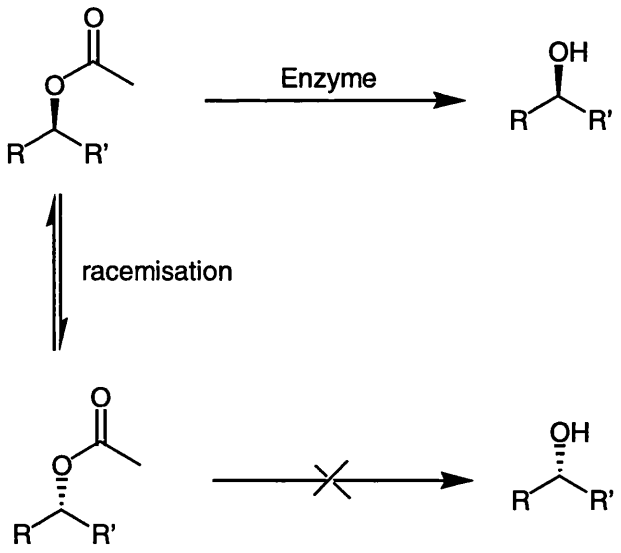
- To overcome problems with the low tolerance of enzymes to high substrate concentrations.

Typically enzymes have been immobilised in three ways, attachment, cross-linking and entrapment. Enzymes are generally attached to either inorganic substances such as silica,[43] organic substances such polystyrene or to themselves in the case of cross-linked enzymes[44-46] They can also be entrapped in bioorganic materials such as the membrane cellulose.[47]

Recently, cross-linked enzyme crystals (CLEC) have been popularised by Altus Biologics[48]. The cross-linking of enzymes such as *Candida cylindracea* lipase has been found to supply a combination of features normally associated with both enzymes (high activity, selectivity, an ability to function under mild reaction conditions and ease of disposal) and heterogeneous catalysts (stability in different environments, recycling). This combined set of properties has made CLEC catalysts extremely useful in organic synthesis.[49,50]

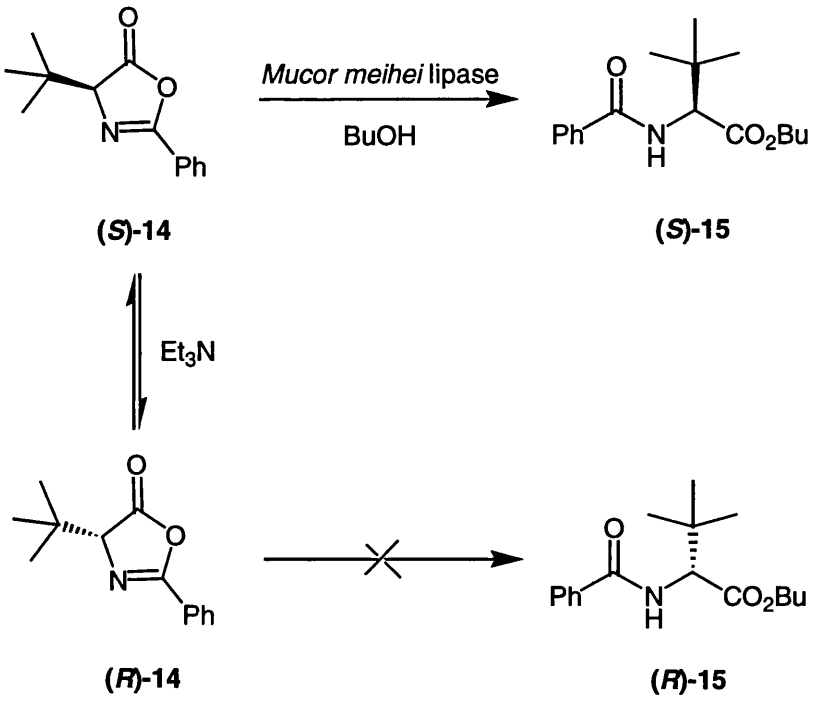
1.7 *Dynamic kinetic resolutions*

A *dynamic resolution* can take place when two enantiomers of starting material are in dynamic equilibrium[51-57] The enzyme will only react with one enantiomer and if the enzyme is constantly being provided with racemic material then 100% of the starting material can be converted into a single enantiomer of product. In order for this methodology to succeed the product of enzymatic conversion must not undergo racemisation as illustrated in **Scheme 6**.



Scheme 6

There are relatively few examples of enzyme mediated *dynamic kinetic* resolutions in the literature but Turner and Winterman[58] have developed an interesting example towards the *dynamic resolution* of oxazolidinones which is described over in **Scheme 7.**

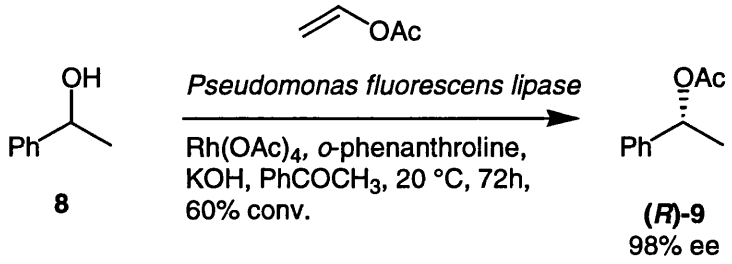


Scheme 7

The acidic α-proton of oxazolidinone **14** is easily deprotonated, which results in rapid racemisation. The enzyme *Mucor miehei* lipase is able to transesterfiy the (S)-enantiomer of oxazolidinone **14** giving the protected amino acid **(*S*)-15** in 99% ee in 94% conversion, which can easily be converted into the natural form of *tert*-leucine.

There are some very successful *dynamic kinetic resolutions* using enzymatic *kinetic resolutions* coupled with chemical racemisation protocols.[59-63] Williams and Backväll have both reported the *dynamic kinetic resolutions* (DKR) of secondary alcohols using the lipase *kinetic resolutions* combined with ruthenium-catalysed racemisation.[64-66]

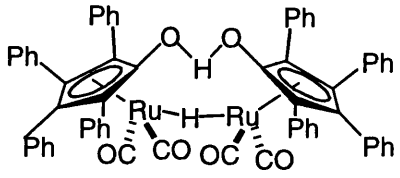
Williams has reported the *dynamic kinetic resolution* of phenethyl alcohol **8** to its corresponding acetate **9** as shown below in **Scheme 8**.



Scheme 8

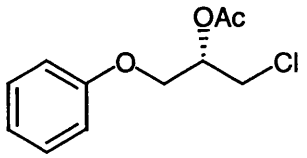
In this example the alcohol is racemised by a metal catalysed transfer hydrogenation mechanism. The coupling of *Pseudomonas fluorescens* lipase and $Rh(OAc)_4$ gave phenethyl acetate in 98% ee at 60% conversion.

Backväll has superseded this resolution using *Candida antartica* lipase and ruthenium catalyst **16** reporting the synthesis of phenethyl alcohol in 99% ee, and 80% yield after 46h.



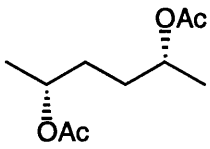
16

His group has also performed the *dynamic kinetic resolution* of a wide range of secondary alcohols[65] and some diols[66] of which the secondary alcohol **17** and diol **18** are examples.



68% yield
79% ee
24h

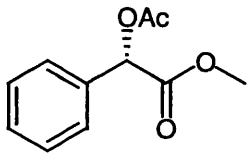
17



63% yield
99% ee
48h

18

Quite recently this methodology has been expanded further to incorporate α-hydroxy esters. The acetate of the methyl ester of mandelic acid **19** has been synthesised in 94% ee and and 80% yield after 48h. In all of these instances **16** is used as a racemisation source.

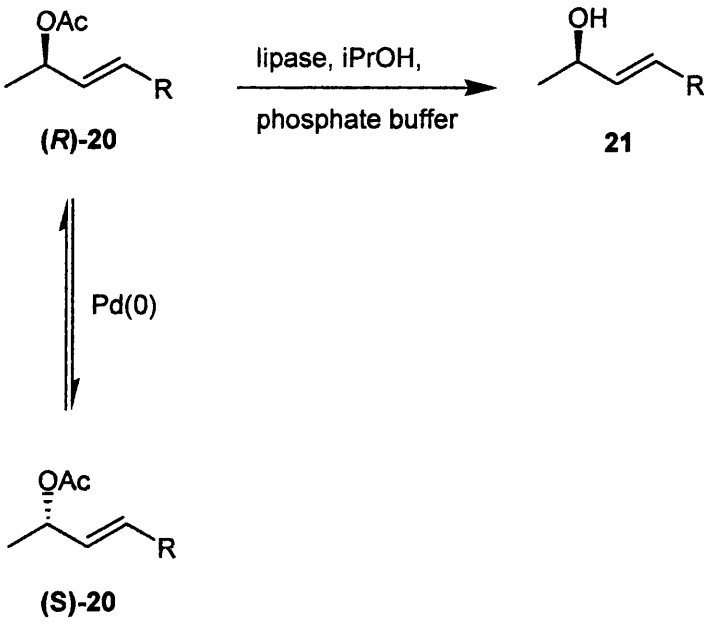


80% yield
94% ee
48h

19

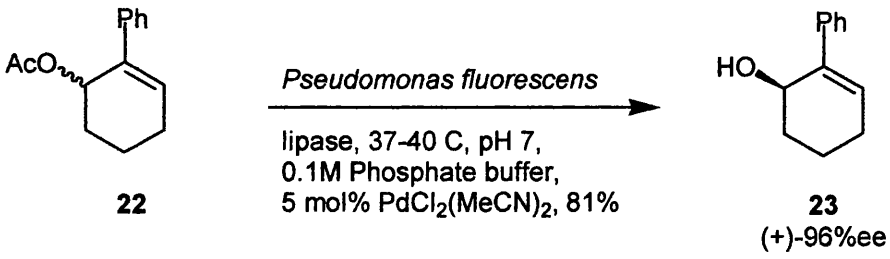
The use of base (triethyl amine) is also discussed as a means of racemisation of **19** but is dismissed as being far less efficient than the ruthenium catalyst **16**.

Allylic acetates such as **20** have also been resolved through *dynamic kinetic resolution* catalysed by biocatalysts working in parallel with racemising agents to give enantiomerically enriched allylic alcohols such as **21**. In this instance palladium(0) is used as a means of racemisation of the allylic acetate as demonstatesd in **Scheme 10.**



Scheme 9

Williams and Allen[67] have pioneered the work in this area showing that the allylic acetate **22** can be resolved in 96 ee% and a 96% yield after 19 days using Pseudomonas fluorescens lipase and palladium dichlorodiacetonitrile as shown in **Scheme 10.**



Scheme 10

More recently, Kim et al[68] who have elaborated to the variety of alkyl allylic acetates, which can be successfully resolved using the methodology developed by Williams et al. have expanded this work.

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Section 2

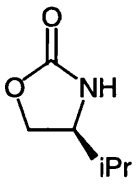
Catalytic Chiral Auxiliaries

2.1 Introduction

This Chapter will describe research carried out towards the *kinetic resolution* of Evans auxiliaries using enzymes and suggest a possible use of this methodology in the development of catalytic chiral auxiliaries.

During the last decade the application of asymmetric synthesis can be widely seen in both academic laboratories as well as in the industrial synthesis of chiral drug and agrochemicals[1,2]. Alongside biocatalysts[3] and metal bound chiral ligands[4-6], chiral auxiliaries[7] complete the synthetic chemists repertoire of asymmetric techniques.

Evans auxiliaries **24**, **25**, **26**, are without doubt, the most heavily used and most synthetically useful auxiliaries designed to date.[8]



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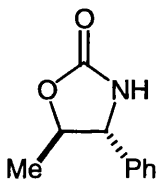

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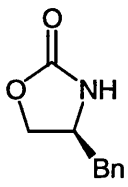
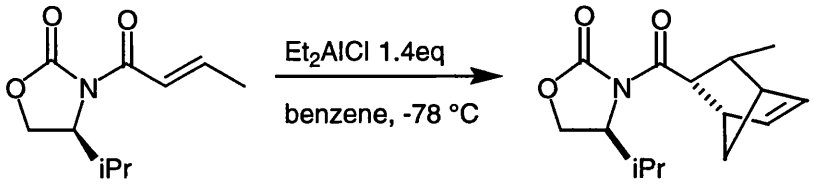


26

The oxazolidinones are known to induce high selectivities in many organic reactions. **Scheme 11** shows their use in an asymmetric Diels-Alder reaction of compound **27** in which the auxiliary controls the *exo:endo* ratio and the diastereomeric excess of the reaction.[9]

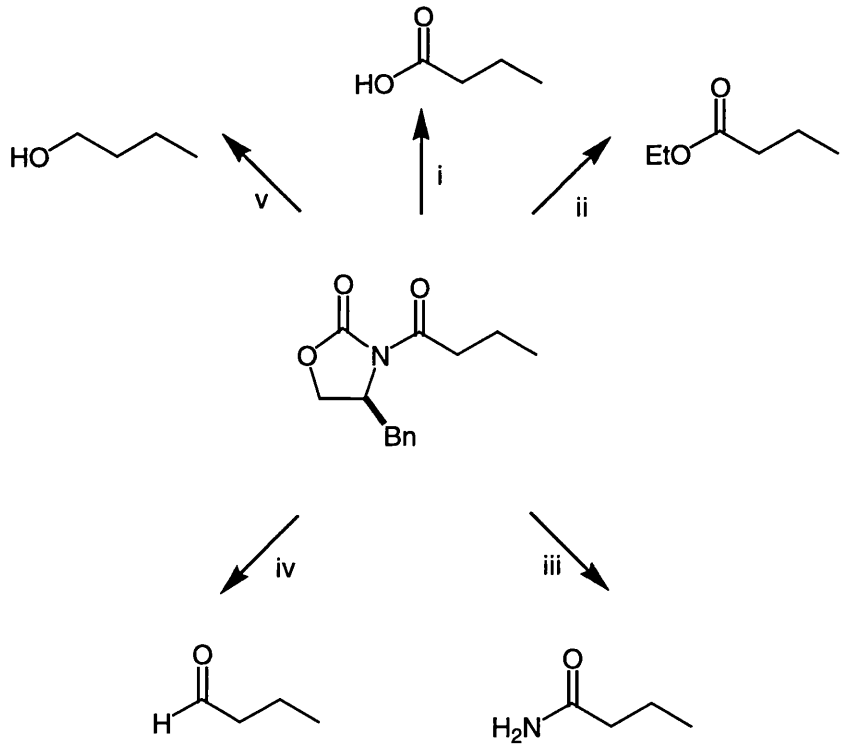


27

78-88%
exo:endo > 98:2
ds > 95:5

Scheme 11

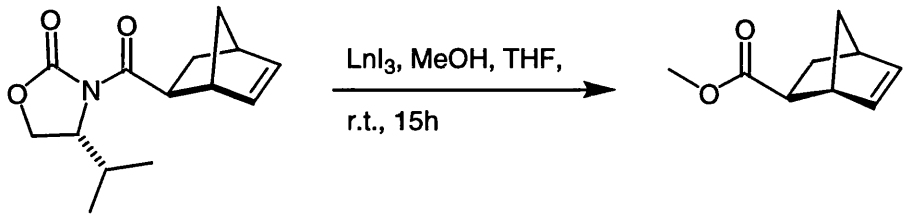
By their nature Evans Auxiliaries are used in stochiometric amounts. Therefore they must be attached prior to reaction and detached afterwards. Attachment is normally carried out by de-protonation of the oxazolidinone and reaction thereafter with an appropriate acid chloride. Detachment of Evans auxiliaries has been reported in a number of ways identified in **Scheme 12.**



Scheme 12

i)KOH, MeOH ii) NaOEt iii) N_2H_4 iv) $LiBH_4$, H_2O v) $LiAlH_4$

Hydrolysis, reduction and ester formation are the most common methods of detachment. **Scheme 13** describes a new mild acting method of auxiliary cleavage reported recently in which Lanthanum iodide is used to transesterify the norbornene adduct **28** to its methyl ester **29**.[10]

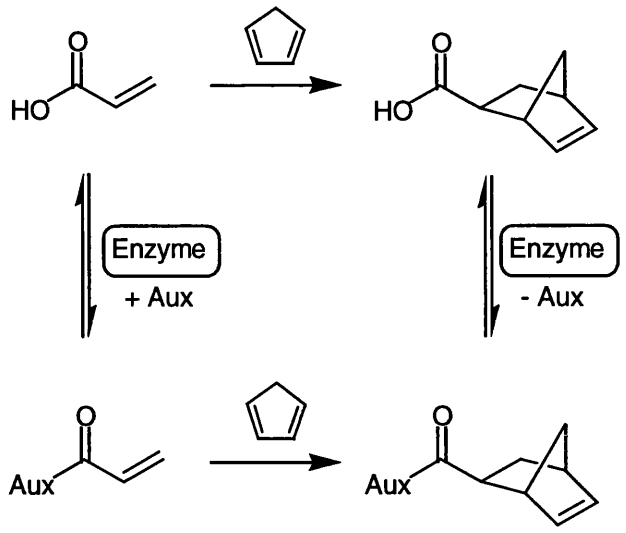


28 **29**

Scheme 13

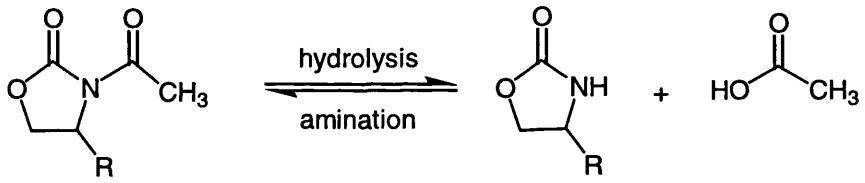
Although Evans auxiliaries have afforded a means to achieve asymmetric synthesis they increase any synthetic route by two synthetic steps. To overcome this our group has been working towards the development of ways in which auxiliaries can be attached and detached in the same reaction vessel they are being used to carry out asymmetric induction[11] as shown in **Scheme 14**.

To achieve this aim we had to identify a reversible reagent, which would be able to both attach and detach the auxiliary. Our minds immediately turned to enzymatic techniques that are well known to work in a reversible fashion.



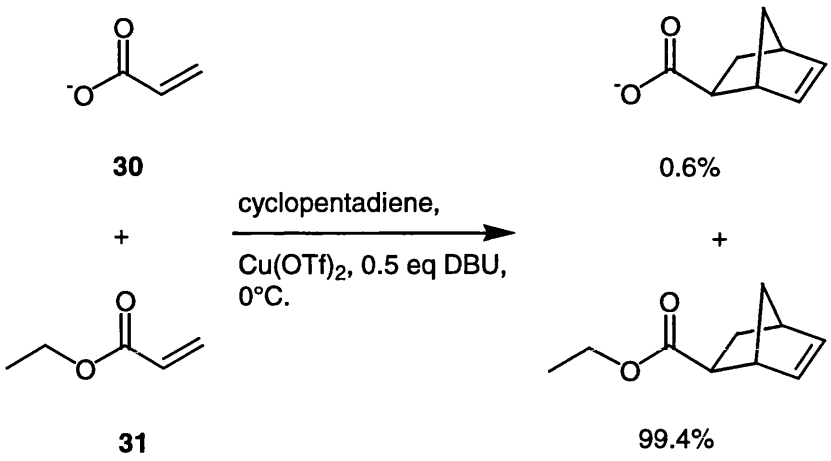
Scheme 14

We envisaged that an enzyme could accomplish both the amination of a suitable acid and also the hydrolysis of its corresponding amide **Scheme 15**.



Scheme 15

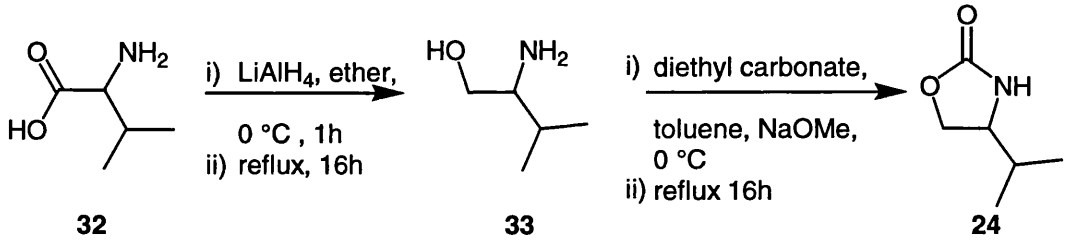
Under suitable conditions we hoped that equilibrium between attachment and detachment could be found. To enable successful catalytic chiral auxiliaries our group has had to find a way in repressing the reaction of non-auxiliary bound acid. Preliminary studies showed that the Diels-Alder reaction of the carboxylate **30** is significantly slower than the Diels-Alder reaction of its ester **31** as shown in **Scheme 16**.[12]



Scheme 16

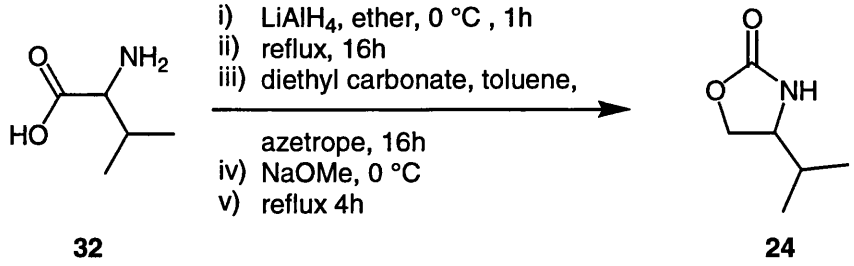
2.2 Enzymatic studies towards the *kinetic resolution* of Evans auxiliaries

Our studies towards the *kinetic resolution* of Evans auxiliaries started with the synthesis of racemic oxazolidinones. The reduction of racemic valine **32**[13] was achieved and the resultant valinol **33** was cyclised using diethyl carbonate[9], giving oxazolidinone **24 Scheme 17.**



Scheme 17

A 'one pot' synthesis of oxazolidinone **24** was also designed. Denis[14] reported a one-pot synthesis of **24** from starting material **32** using phosgene as a means of cyclisation. The idea was modified using diethyl carbonate a much milder reagent as shown in **Scheme 18.**

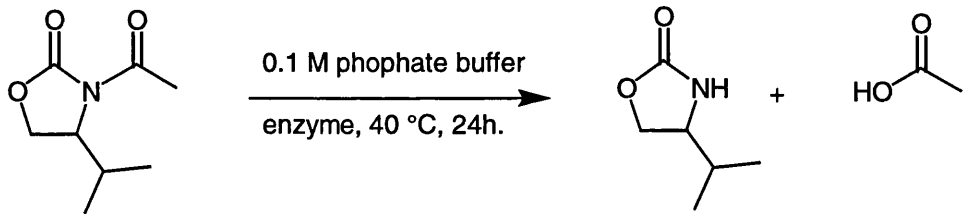


32 **24**

Scheme 18

Although this synthesis was found to be successful, the yield of 54% was mediocre. More efficient mechanical stirring may increase yields by breaking down the aluminium salts formed when $LiAlH_4$ is quenched. Acylation of oxazolidinone was achieved by deprotonation of the auxiliary and quenching the anion formed with the relevant acid chloride.[15]

The hydrolysis of oxazolidinone **24** was screened for enzyme candidates as shown in **Scheme 19**. Generally, lipases were employed but a number of proteases and esterases were also tried.[16] The standard conditions for these reactions were pH 7.5 phosphate buffer, 40 °C with a reaction time of 24h. A control reaction using no enzyme was routinely carried out and non-enzymatic or 'background' reactions were not observed. *Pseudomonas fluorescens* lipase and *Candida antarctica* lipase were the only enzymes to hydrolyse the amide bond in **34**. In both cases the hydrolysis proceeded with no selectivity for one enantiomer over the other. All conversions were confirmed by proton NMR of the crude reaction. We examined the ratio of the NMR signal corresponding to the CH_2 on the oxazolidinone relative to the CH_3 group of the acetate.

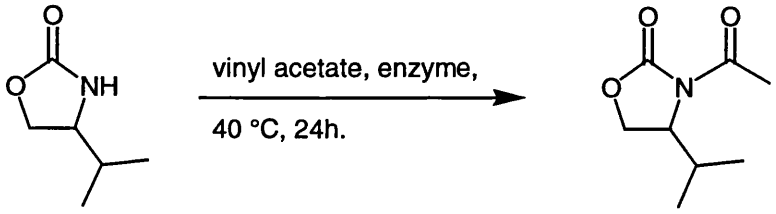


34 **24**

Pseudomonas fluroscens lipase	25% conv.
Candida antartica lipase	37% conv.

Scheme 19

Even though our hydrolysis studies hadn't yielded a successful kinetic resolution of the acyl-oxazolidinone **34** as we hoped the acylation of oxazolidinone **24** was attempted as shown in **Scheme 21**. Thus treatment of oxazolidinone **24** with vinyl acetate gave the acetate **34**.



24 **34**

Scheme 21

In the first example shown in **Table 1** vinyl acetate as both acyl donor and solvent giving a 20 % conversion of oxazolidinone **24** to its acetate **34** after 24h. Reducing the acyl donor concentration to 50% v/v slowed down the reaction to such an extent that 0% yield was observed after 24h. A similar result was observed when 3 equivalents of vinyl acetate was employed as shown in **Table 1**

Acyl Donor	Conversion (%)
Vinyl acetate (100% v/v)	20
Vinyl acetate (50% v/v)	0
3 eq vinyl acetate	0

Table 1: The acylation of evans oxazolidinone using *Candida antarctica* lipase.

A range of solvents were tried to investigate there effect upon the acylation of oxazolidinone **24** to its acetate **34**. *t*BuOMe and Methanol gave the most promising results with 38% and 24% conversion of oxazlidionone to acetate after 24h. Conversely, reaction carried out in toluene and acetone showed extremely poor conversions as shown in **Table 2**.

Solvent	Conversion (%)
MeOH : vinyl acetate	24
Toluene : vinyl acetate	<3
Acetone : vinyl acetate	<1
t-BuOMe : vinyl acetate	38
MeOH (3 eq) in vinyl acetate	0
Toluene (3 eq) in vinyl acetate	0
Acetone (3 eq) in vinyl acetate	<1
t-BuOMe (3 eq) in vinyl acetate	<3

Table 2: Asolvent study of the acylation of oxazolidinone **24** catalysed by *Candida antarctica* lipase

Both vinyl acrylate and trifluoroethyl acrylate were employed as acyl donors and solvent but no reaction was observed. Variation of temperature showed that that the reaction worked best at 40 °C, could be observed at 50 °C but not 60 °C. It also showed that Boeringer Mannheim's *Candida antarctica* lipase was more effective than the equivalent enzyme supplied by Novo Nordisk as shown in **Table 3**. With an

83% conversion of oxazolidinone to its acetate in 24h a morked improvement on previous results.

Enzyme	Temp (°C)	Conv (%)
Candida antarctica lipase Novo Nordisk	40	<5%
Candida antarctica lipase Boerhinger Mannheim	40	83%
Candida antarctica lipase Boerhinger Mannheim	50	54%
Candida antarctica lipase Boerhinger Mannheim	60	-

Table 3: A temperature study of the acylation of oxazolidinone **24** using *Candida antarctica* lipase

We attributed the increase in conversion to high activity of the boeringer mannhiem enzymes used.

2.3 Conclusion

We have found that the scope of the reaction is severely limited to acetate formation, the reaction times are slow and no *kinetic resolution* is observed. We therefore concluded that the manipulation of Evans auxiliaries with lipases is neither facile nor flexible enough to be useful in the development of catalytic chiral auxiliaries

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16 in '*Mucor javanicus*, *Geotrichum candida*, *Candida rugosa*, *Alicalgenes species*, *Pseudomonas cepacia*, *Pseudomonas species*, Liproprotien, Hog pancreas. lipase and Horse liver esterase, alpha-chymotripsin, *Aspergillus Q* protease, *Bacilus lich.* protease,'.

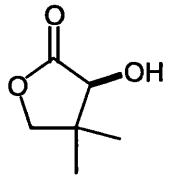
Section 3

The *Kinetic Resolution* of Pantolactone Chiral Auxiliary

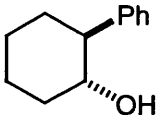
3.1 Introduction

Further to our investigations into catalytic chiral auxiliaries we carried out research towards the *kinetic resolution* of Pantolactone chiral auxiliary through, acylation, esterification and transesterification will be discussed in this chapter.

A literature search cross-referencing the major auxiliaries with biocatalysts gave some promising leads, which indicated to date, the type of chiral auxiliaries that have been manipulated using an enzyme. We found that pantolactone **35** Whitesell's **36** chiral auxiliaries have been resolved using various enzymes.[1-4]



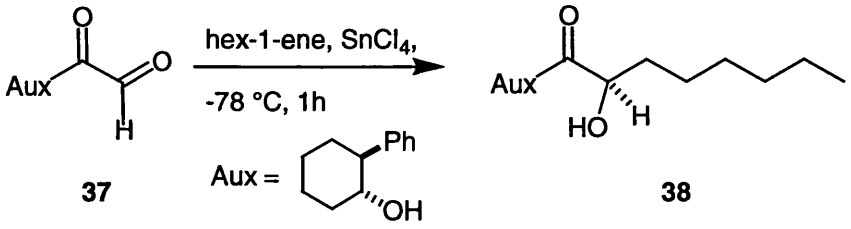
35



36

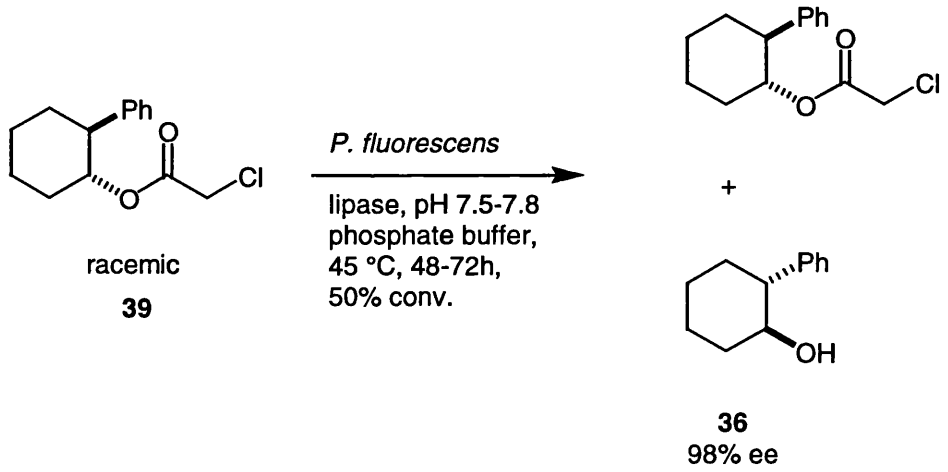
Both Whitesell's and Pantolactone auxiliaries are secondary alcohols, unlike Evans auxiliary in structure but akin to phenethyl alcohol, a very popular substrate for enzymatic *kinetic resolutions*.[5] As with the most successful *kinetic resolutions* of phenethyl alcohol these auxiliaries are resolved by the formation or hydrolysis of their corresponding acetates using biocatalysts from the lipase family.

Whitesell's auxiliary was developed to parallel 8-phenyl menthol[6] and has been successfully been used to control ene reactions.[7] **Scheme 21** Shows that the α-ketoaldehyde **37** was converted into the α-hydroxy aldehyde **38** in the presence of tin chloride. The reaction proceeded in 75% and 98% enantioselectivity.



Scheme 21

Pseudomonas fluorescens lipase has been reported to facilitate the resolution of Whitesell's auxiliary from its chloroacetate in 98% ee at 50% conversion by NMR.[1] This process has been refined and published as an 'organic synthesis' preparation, which includes the protocol for the racemic synthesis of **36** as well as its *kinetic resolution.*[2,8] The kinetic resolution of Whitesell's auxiliary is shown in **Scheme 22.**

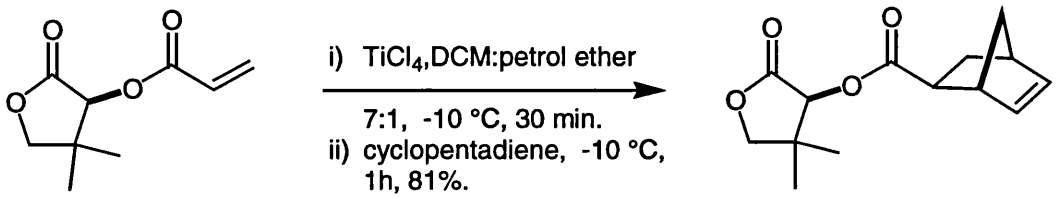


Scheme 22

Further investigations towards the manipulation of Whitesell's auxiliary have been carried out within our group but will not be discussed here.[9]

In 1985 Helmchen[10] et al found that pantolactone chiral auxiliary could effectively be used to perform enantioselective Diels-Alder reactions. Helmchen reported that

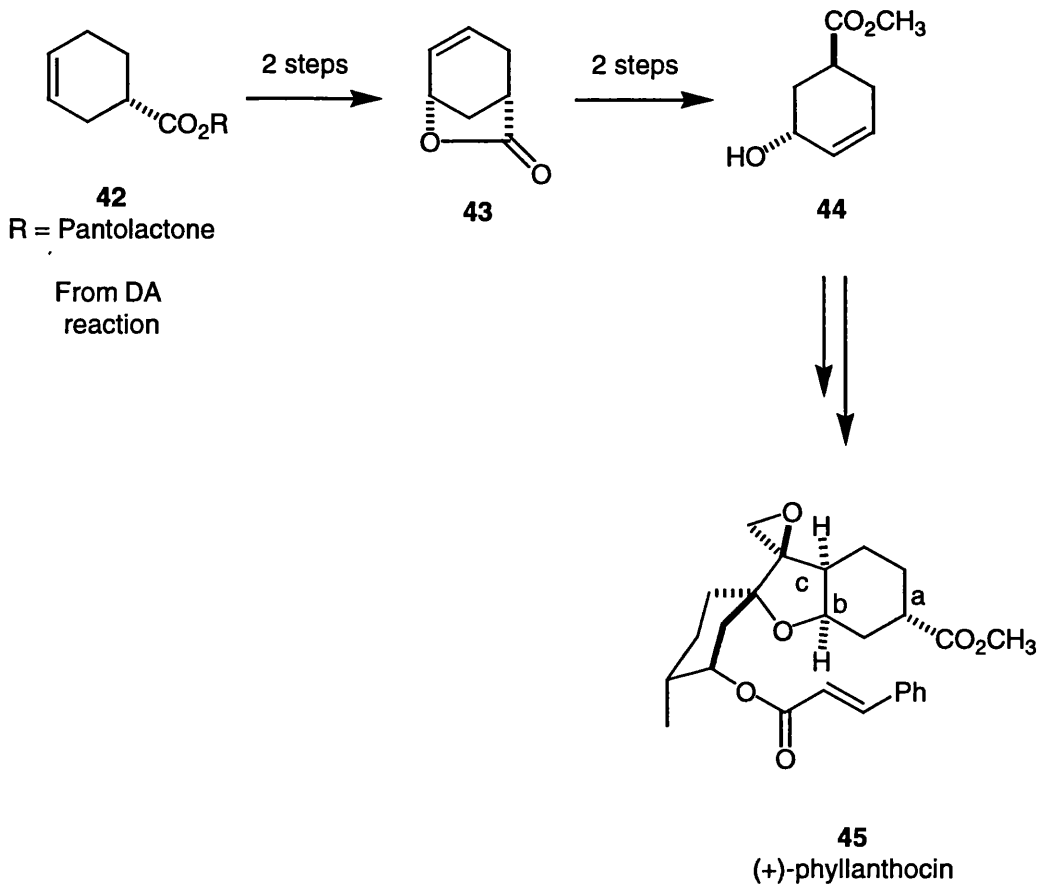
Pantolactone acrylate could undergo a Diels-Alder reaction in the presence of $TiCl_4$ giving excellent *endo* selectivities as well as high diastereoselectivities. **Scheme 23** shows the Diels Alder reaction of (*R*)-pantolactone acrylate **40** with cyclopentadiene in the presence of titanium chloride. The reaction yielded 97% of the *endo* diastereoisomer **41**.



(*R*)-40 **41** 97:3 de

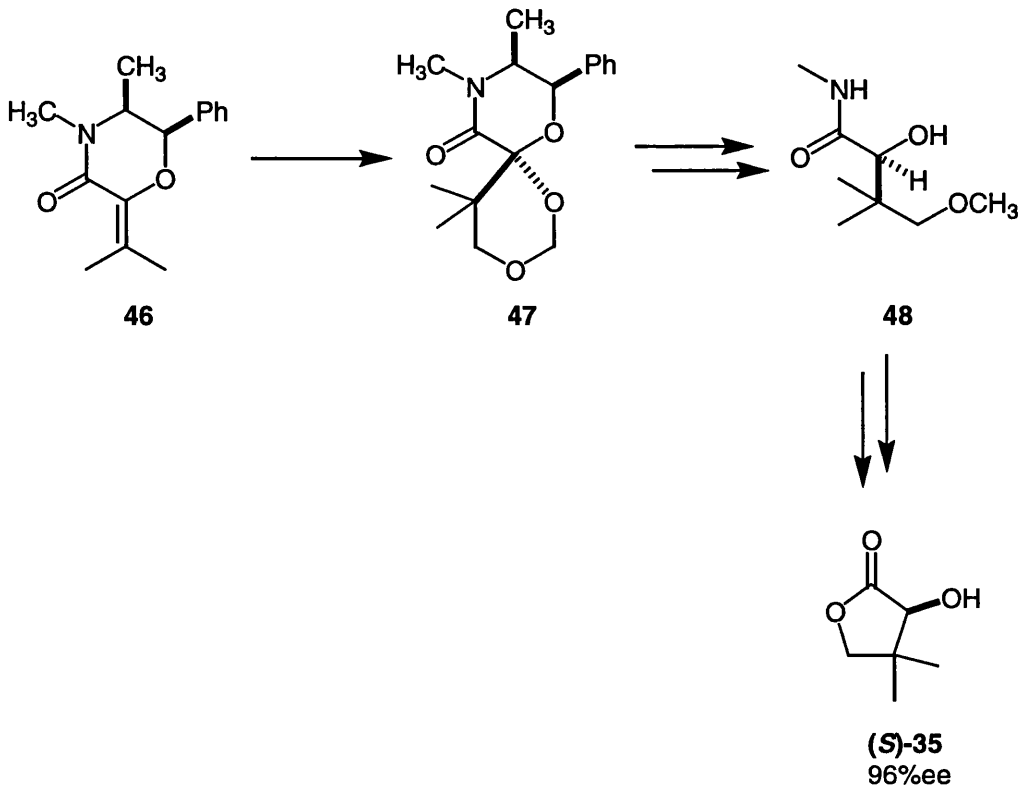
Scheme 23

It is therefore not surprising that, a number of synthetic groups have used this methodology in total synthesis. For example, Trost has used pantolactone to synthesise a number of key chiral centres in the natural product (+)-phyllanthocin **45**[11] as shown in **Scheme 24**. The chiral auxiliary allowed chiral centre 'a' to be formed through a Diels-Alder reaction and this chiral centre was used to induce chirality at centres 'b' and 'c'.



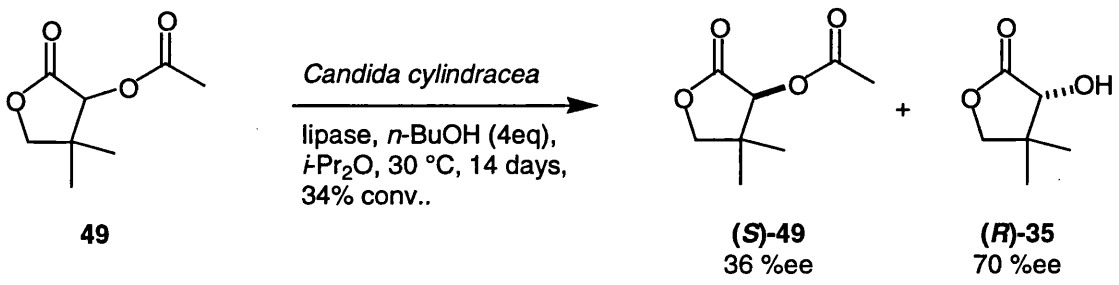
Scheme 25

Considering the wide use of Pantolactone it is also not surprising that there are several publications highlighting its synthesis and resolution. Recently, Pansare[12] found that the Prins reaction of the chiral alkylidene morpholinone **46** derived from (1R,2S)-ephedrine and 3-methyl-2-oxobutanoic acid proceeds with good diastereoselectivity to generate the spiro bis-acetal **47**. Lewis acid mediated diastereoselective reductive cleavage of the spiro acetal and subsequent removal of the ephedrine portion generates **48**, which is readily converted into (*S*)-Pantolactone (***S*)-35** in six high yielding steps and in 96% ee as shown in **Scheme 25**.



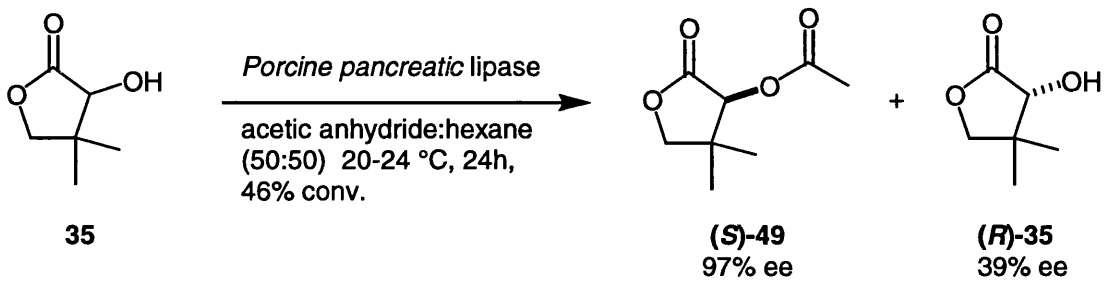
Scheme 25

The *kinetic resolutions* of Pantolactone using biocatalysts provide the best methods of resolving the auxiliary to date. **Scheme 26** illustrates the first account of the resolution of Pantolactone using *Candida cylindracea* lipase to transesterify racemic Pantolactone acetate **49** in 34% conversion giving pantolactone acetate **(*S*)-49** in a modest 36% ee at after 14 days.[4]



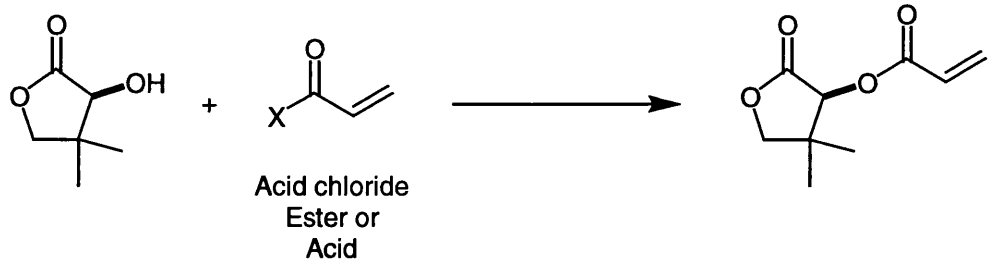
Scheme 26

Eight years later in 1997 Gamalevich published a highly successful *kinetic resolution* using *Porcine pancreatic* lipase. **Scheme 27** shows that racemic Pantolactone **35** underwent a *kinetic resolution* through the formation of its acetate[3] in 97% ee and 46% conversion thus resolving the remaining pantolactone in 39% ee.



Scheme 27

But how useful are these resolutions for synthetic organic chemists? From a synthetic point of view neither pantolactone nor its acetate are immediately of use as these substrates are not suitable precursors to a Diels-Alder reaction. Typically enantiomerically pure Pantolactone is added to an acrylic acid, acid chloride or ester as shown in **Scheme 28.**[10]

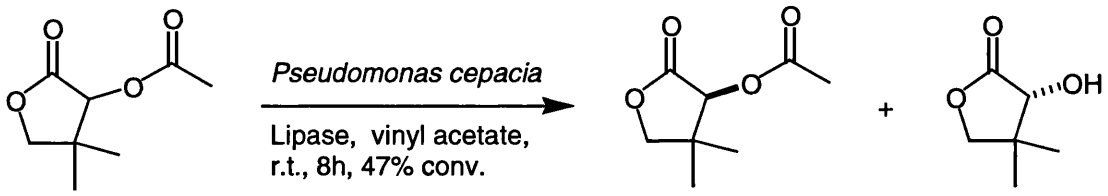


Scheme 28

It became clear to us that if Pantolactone could be resolved as its acrylate then this process would not only be a method of resolving Pantolactone but a way of doing so, providing a suitable precursor for Helmchen's methodology.

3.2 The *kinetic resolution* of pantolactone by the formation of Pantolactone acetate.

Although, our final goal was to synthesise enantiomerically pure pantolactone acrylate our initial studies were aimed towards the *kinetic resolution* of pantolactone by the acetate formation. Following Gamalevich[3] and Bevinakatti[4] the lipases *Candida cylindracea* (CLEC Altus-17), *Porcine pancreatic* (Fluka) and *Pseudomonas cepacia* lipase (CLEC Altus-20) were screened along with *Candida antarctica* lipase (Novozym 435). We were particularly interested in using CLEC's as they have proved to be very efficient in the resolution of phenethyl alcohol previously within our group.[13] Vinyl acetate was employed as an acyl donor rather than acetic anhydride as this too had proven to be highly successful in work previously done in the Williams group.[8]



49 **(*S*)-49** 99 %ee **(*R*)-35** 99 %ee

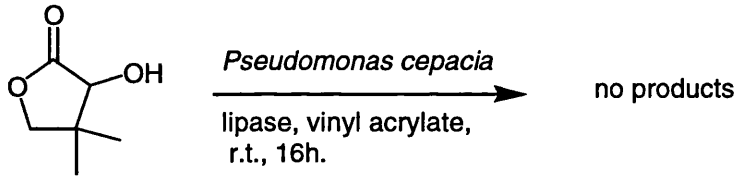
Scheme 29

We carried out the reaction described above in **Scheme 29** finding that after 24h TLC analysis showed that *Pseudomonas cepacia* lipase CLEC had catalysed the acylation

of pantolactone. This reaction was repeated (100mg Pantolactone, 20mg enzyme, 1 mL solvent) giving (*S*)-Pantolactone acetate in 99% ee and a 48% conversion after 8h at room temperature.

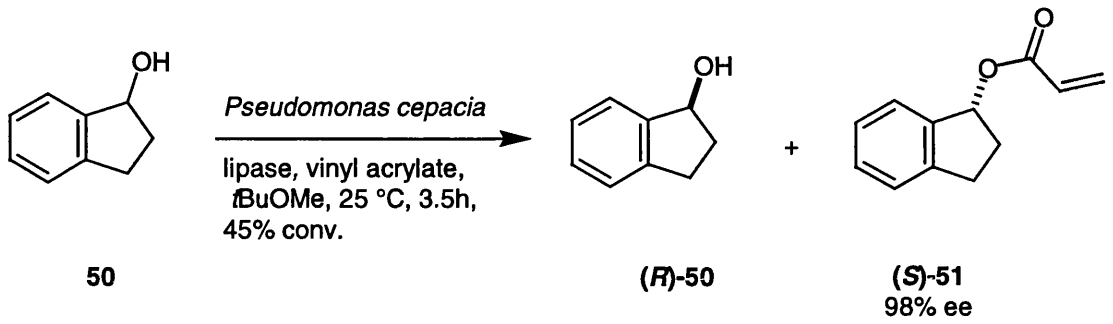
3.3 The *kinetic resolution* of Pantolactone by the formation of Pantolactone acrylate.

Upon repeating the procedure shown in Scheme 30 using vinyl acrylate rather than acetate the *kinetic resolution* of Pantolactone did not occur as shown in **Scheme 30**.



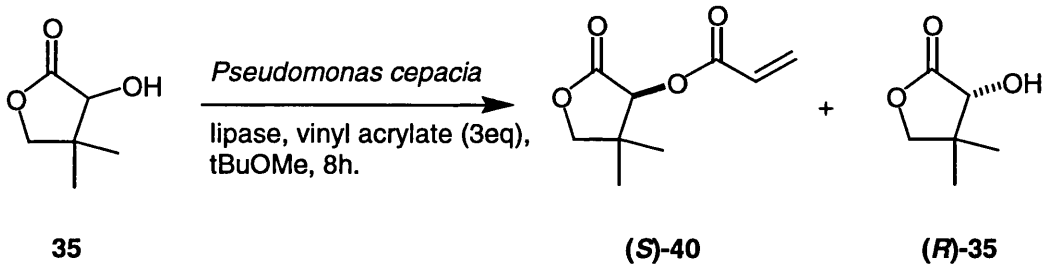
Scheme 30

We concluded that either vinyl acrylate or its polymerisation inhibitor might have denatured the enzyme. The reactivity of the acrylate versus the acetate was also questioned, was vinyl acetate electrophilic enough? Unsure exactly why the reaction hadn't worked, the ISIS base-biocatalysts database was searched for examples of acrylate resolution of secondary alcohols. To date, only two such examples[14,15], were found one of which is highlighted over in **Scheme 31**.



Scheme 32

Klibanov et al have used both vinyl acrylate and trifluoroethyl acrylate to resolve a number of secondary alcohols and amines. Indanol **50** was resolved using trifluoroethyl acrylate 3eq in *t*BuOMe at 25 °C giving 98% ee of **(*R*)-50** at 45% conversion. Upon repeating the acrylation of pantolactone under the conditions of Klibanov we were delighted to find pantolactone could be resolved in good ee% as shown in **Scheme 32**, and over in **Table 4**.



Scheme 32

Acyl Donor	Pantolactone Acrylate ee%	Pantolactone ee%	Conv. (NMR)
Vinyl acrylate	88	97	56
Trifluoroethyl acrylate	91	32	24

*50 mg Pantolactone, vinyl acrylate 3 eq, 1 mL *t*BuOMe, 10% w/w enzyme, 17h, r.t.

Table 4: The acylation of pantolactone using *Pseudomonas cepacia* lipase.

Both vinyl acrylate and trifluoroethyl acrylate were successful acyl donors giving 88% ee at 56% conversion and 91% ee at 24% conversion respectively. In doing this we have proved that acrylate **40** can be formed under enzyme catalysis. Klibanov conditions had given us a good result although the enantioselectivity of the acrylates was lower than those found for acetate. Subsequently, Jochen Zimmermann an ERASMUS student who joined the Williams group completed an optimisation study of acrylate *kinetic resolution* to try to improve the enantioselectivity of the transformation such to parallel the high selectivities found in the acetate formation. The results are reviewed below.

3.4 The optimisation of the enzymatic resolution of Pantolactone acrylate.

Optimisation of the solvent type and acyl donor concentration were undertaken. A range of ethers, alkanes, alcohols, ketones and chlorinated solvents were tried. While the ethers, tertiary butyl methyl ether, diethyl ether and diisopropyl ether comprehensively gave the best results, toluene, acetonitrile, acetophenone and acetone were also successful. **Table 5** shows that tBuOMe gave the best enantioselectivity at the best conversion.

Solvent	Pantolactone acrylate % ee	Pantolactone % ee	Conversion %	E
tBuOMe	95	46	32	61
diethyl ether	94	38	24	46
iPr_2O	92	61	39	44
Acetophenone	93	17	14	32
Acetone	94	9	3	35
Toluene	9	14	90	1.3

100 mg Pantolactone, vinyl acrylate 3 eq, *t*BuOMe 1 mL, enzyme 10% w/w, 24h, r.t.

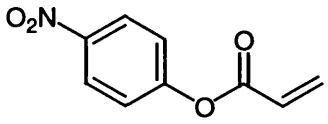
Table 5: A solvent study of the *kinetic resolution* of pantolactone to it acrylate catalysed by *Pseudomonas cepacia* lipase.

Acyl donor concentration was also investigated as shown over in **Table 6**. The higher concentration of acyl donor the better conversion observed, with little effect on the selectivity of the enzyme. For example, in reactions where 0.5 equivalents of vinyl acrylate were used pantolactone acrylate is formed in 95% ee but only 7% conversion. Alternatively when 5 equivalents of vinyl acrylate are employed pantolactone is resolved by forming it acrylate in 85% ee and 56% conversion. We have shown that the concentration of acyl donor effects both the conversion and the enantioselectivity of the reaction and had found our initial conditions to be the most suitable.

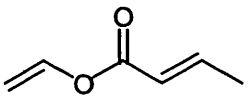
Equivalents of acyl donor	Pantolactone acrylate % ee	Pantolactone % ee	Conversion NMR	E
0.5	95	5	70	7
1.0	94	31	27	27
3.0	89	83	50	50
5.0	85	94	56	56

Table 6: A study investigating the role of acyl donor concentration on the kinetic resolution of Pantolactone to its acrylate catalysed by *Pseudomons cepacia* lipase.

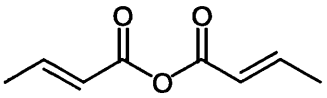
Continuing our methodology study a further three acyl donors **52, 53, 54,** were tested but none showed reaction. This was particularly disappointing in the case of the crotonates **53** and **54** as their lack of reactivity highlighted a limit to the methodology.



52



53



54

Finally a temperature study was performed and the results are recorded in **Table 7.** Comparable conversions and enantioselectivities were reported for *kinetic resolutions* carried out between room temperature and 35 °C but there after at temperatures higher than 35 °C polymerisation of the acyl donor resulted. This was disappointing, as CLEC enzymes have been reported to be stable up to 80 °C.

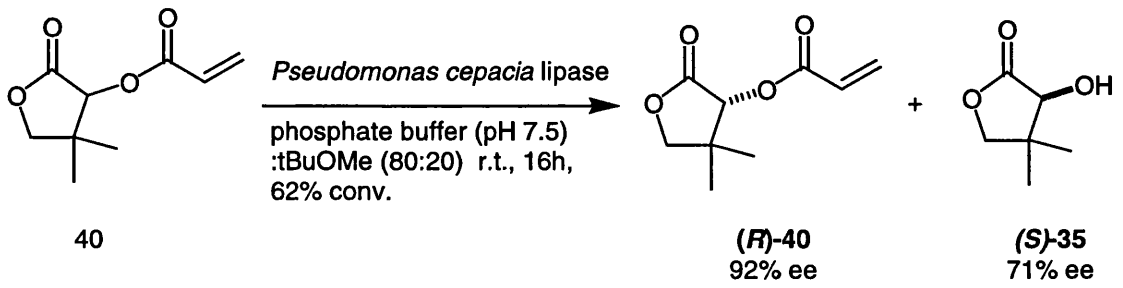
Temperature °C	Pantolactone acrylate % ee	Pantolactone % ee	Conversion NMR	E
25	89	87	50	48
30	87	90	53	44
35	88	79	48	37
40	Polymerisation of acyl donor			
50				

Table 7: A temperature study of the *kinetic resolution* of pantolactone to it acetate catalysed by *Pseudomonas cepacia* lipase.

From this optimisation study we concluded that our initial conditions were almost optimum!! Never the less it was discovered that crotonates were not viable acyl donors and that the reaction could not be carried out at temperatures over 35 °C.[16]

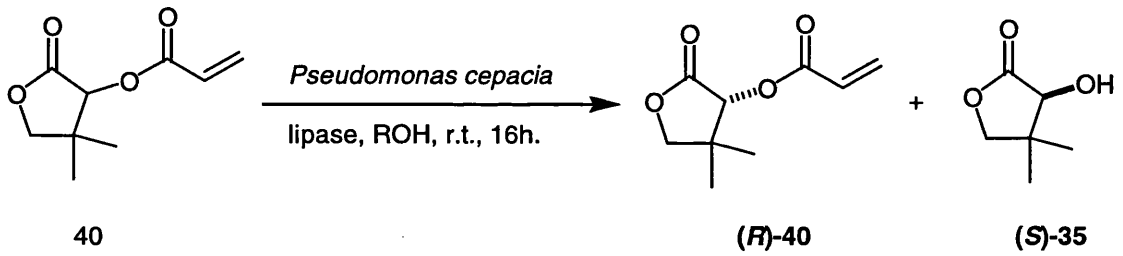
3.5 The hydrolysis and transesterification of Pantolactone acrylate

After a successful methodology study involving the *kinetic resolution* of pantolactone through acrylate formation, the hydrolysis and transesterification of Pantolactone were investigated. As the solvent *t*BuOMe, temperature 25 °C and the concentration of 50mg per mL had been proved to be successful in bond formation they have continued to be used in our hydrolysis and transesterification experiments. **Scheme 33** describes the hydrolysis of pantolactone acrylate **40** we carried out.



Scheme 33

The hydrolysis of racemic pantolactone acrylate yielded enantiomerically enriched pantolactone acrylate in (92% ee) at 62% conversion, which was comparable to the enantioselectivities reported in our previous acrylation experiments. Repeating this experiment in the presence of an alcohol source rather than buffer showed that Pantolactone could be transesterified using a number of different alcohols as solvents as shown in **Scheme 34** and **Table 8.**



Scheme 34

Pantolactone acrylate could be resolved in 74% ee using *Pseudomonas cepacia* lipase and MeOH as a solvent. Other alcohols were tried but in all cases the enantioselectivities for these reactions were poor. This was especially true in the case of the deactivated alcohol, trifluoroethanol that gave little selectivity and almost no conversion as shown in **Table 8.**

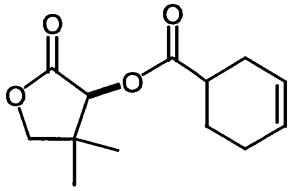
Solvent	Acrylate ee%	Pantolactone ee%	Conv. (NMR)	E
MeOH	14	74	23	7.7
EtOH	11	49	28	3.3
i-PrOH	10	44	5	2.8
CF_3CH_2OH	8	4	<5	1.2
MeOH (10eq) in t-BuOMe	37	76	35	10

Table 8: The transesterification of pantolactone acrylate catalysed by Pseudomonas cepacia lipase.

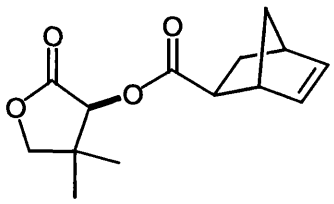
Upon reflection, better results may have been obtained in the transesterification experiments as it has been shown that longer chain alkanes such as C_4, C_6 and C_8 alcohols have generally provided better selectivities over C_1 and C_2 alcohols.

3.6 The Enzymatic Cleavage of Pantolactone from Diels-Alder Adducts.

As much as we were delighted that *Pseudomonas cepacia* lipase CLEC hydrolysed Pantolactone acrylate in good ee% we knew this wasn't as synthetically useful as the hydrolysis of Diels-Alder products **55** and **41**.

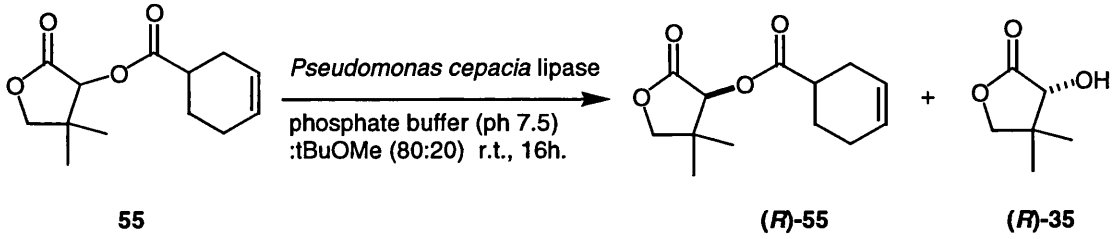


55



41

To date there are no known enzymatic resolutionsof cyclohexene esters. Never the less we set out to investigate the hydrolysis of cyclohexene carboxylic acid pantolactone ester **55**, **Scheme 35** outlines the ideal scenario for the hydrolysis. Pantolactone was observed by TLC analysis of our preliminary reaction at 16h. Further analysis of the crude reaction mixture by NMR showed a 22% conversion of ester **55** to alcohol **(*R*)-35.**



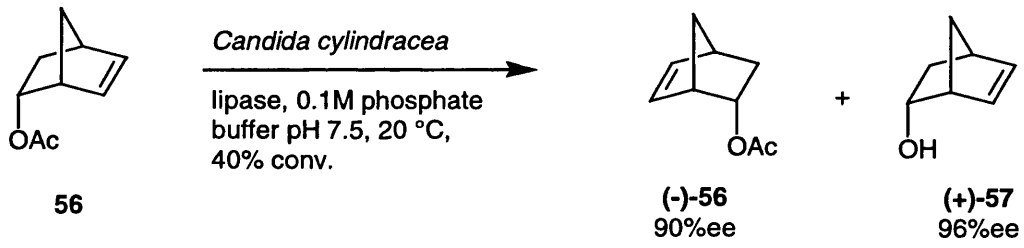
55 (*R*)-55 (*R*)-35

Scheme 35

Chiral Gas Chromatography analysis showed traces of enantio-enriched pantolactone (91% ee) an encouraging result. The analysis of the remaining ester **55** by chiral gas chromatography was found to be problematic. **Figure 1** shows that the best resolution of the ester **55** we have achieved, the chromatograph shows the compound to be represented by three peaks rather than the expected four. The GC chiral separation of the methyl ester of norbornene carboxylic acid was also attempted but as with the ester **55** the two enantiomers of the methyl ester of norbornene carboxylic acid weren't successfully resolved **Figure 1.**

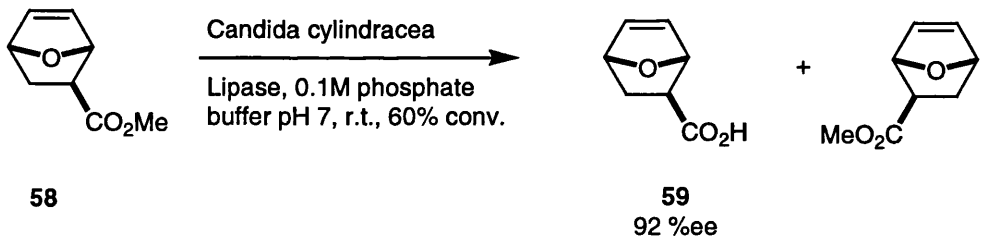
Figure 1: The chiral gas chromatographic analysis of the ester **55**

Our attentions now turned to the enzymatic hydrolysis of the norbornene adduct **41**. In this instance there was literature precedence for the manipulation of such substrates using an enzyme. Greingel has reported that *Candida cylindracea* lipase and *Pseudomonas species* lipase have been found suitable in the resolution of bicyclic esters and alcohols.[17,18] He reported the multi gram preparation of (+)-norbornenol **57** was achieved through the kinetic resolution of its acetate using the enzyme *Candida cylindracea* lipase giving 96% ee of the norbornenol **57** in 40 % conversion as shown in **Scheme 36**.



Scheme 36

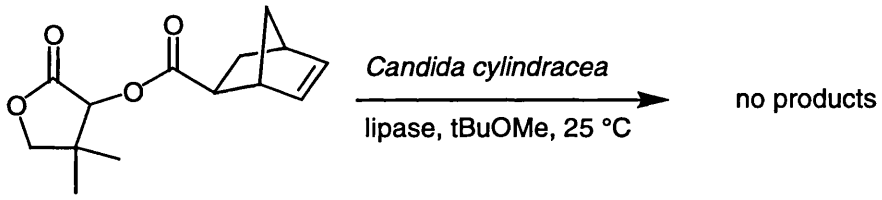
Interestingly, enantio-differentiation by the enzyme was only observed in the case of *endo*-norbornenyl acetate but not in the case of *exo*-norbornenyl acetate. Alternatively Kiessling has shown the hydrolysis of the methyl ester of carboxylic acid **58** could be achieved using *Candida cylindracea* lipase[19] yielding the acid **59** in 92% ee and 60% conversion as shown in **Scheme 37**.



58 **59** 92 %ee

Scheme 37

Norbornene ester **41** was treated under our hydrolysis protocol with both *Candida cylindracea* lipase, *Pseudomonas cepacia* lipases and *Candida antartica* lipase. After 24h Pantolactone was not observed by TLC or found in further chiral gas chromatography experiments as shown in **Scheme 38.**

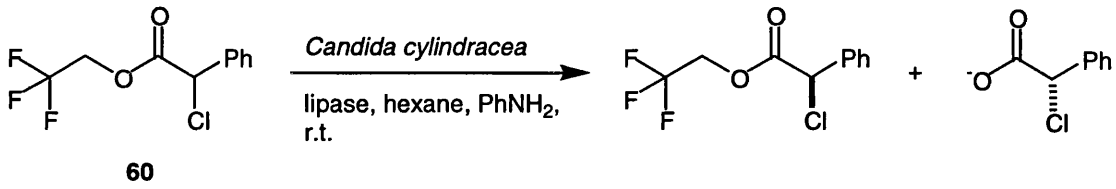


41

Scheme 38

This was both disappointing and surprising as neither *Pseudomonas cepacia* lipase previously shown to be efficient in the manipulation of pantolactone or *Candida cylindracea* lipase reported to be excellent in manipulating norbornene esters and acids had been successful in catalysing the reaction shown in **Scheme 38.** With these precedents we found it difficult to conclude that **41** was not a substrate for either *Candida cylindracea* lipase or *Pseudomonas cepacia* lipase, there must be some other factor. Our hydrolysis conditions were fairly 'standard' and had shown good results in previous experiments this led us to consider other probable causes. We came to postulate that it was possible that the reverse reaction was more prevalent than

hydrolysis, such that the equilibrium of the reaction in **Scheme 38** was over towards starting materials rather than products. If this was the case then could we push the equilibrium over to 'products' by using an idea highlighted by Gotor[20] shown in **Scheme 39**.

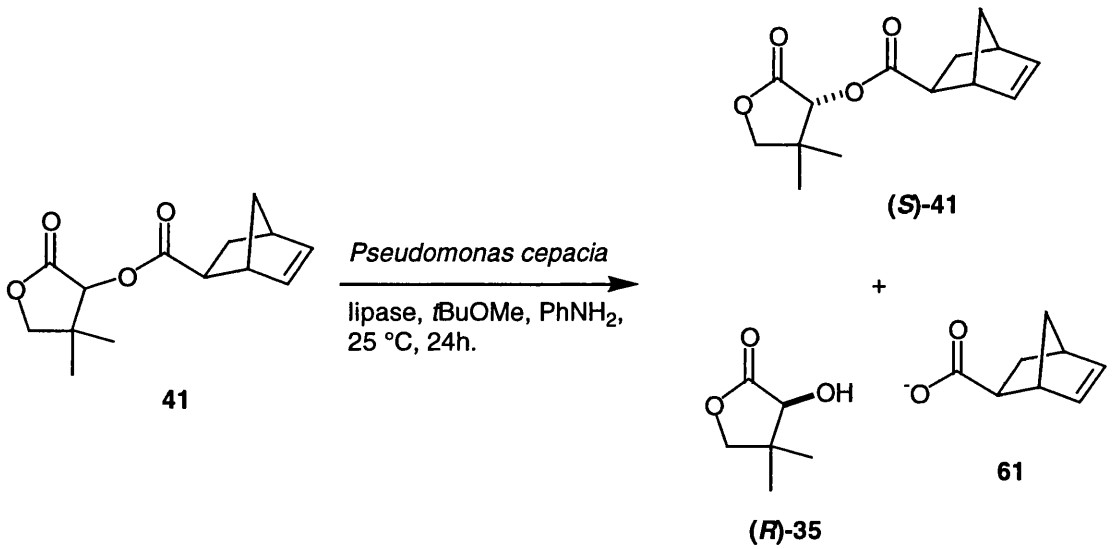


60

Scheme 39

Gotor used aniline to control the hydrolysis of the base sensitive compound **60** using almost anhydrous reaction conditions. In fact he could facilitate the hydrolysis of **60** adding as little as 15 μl of water.

We repeated out previous experiment using *Pseudomonas cepacia* lipase but this time added 1.1 eq of aniline to the reaction as shown in **Scheme 40**.



41

(*S*)-41

61

(*R*)-35

Scheme 40

After 24h we were excited to find traces of Pantolactone by TLC. The reaction was repeated and preparative TLC undertaken to gravimetrically determine a conversion of ester **41** to alcohol **35**. Chiral gas chromatographic analysis of the Pantolactone band recovered from preparative TLC analysis showed Pantolactone as a single enantiomer. The results from **Scheme 40** are reported below in **Table 9**.

	Yield	**ee%**
Pantolactone **35**	38	100
Ester **41**		83

Table 9: Chiral GC and gravimetric analysis of the hydrolysis of **41** using Candida cylindracea lipase in the presence of analine.

From these results we were almost certain that the hydrolysis of the norbornene ester **41** by *Pseudomonas cepacia* lipase was highly selective. But, we needed to analyse the diastereomeric excess of the remaining norbornene starting material to complete our study. A lengthy chiral gas chromatographic optimisation concluded that all eight diastereoisomers of **41** (4 *exo* and 4 *endo* isomers) could be observed on a cyclo-dextrin column at 155 °C (see appendices I & II). Figure 2 represents 4 of the either the *exo* or *endo* isomers.

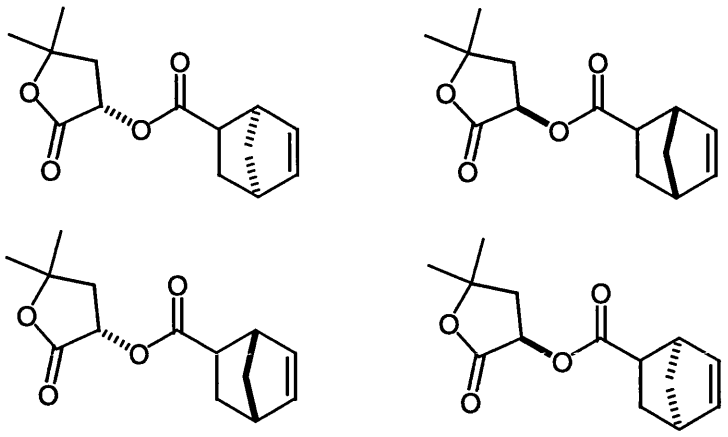
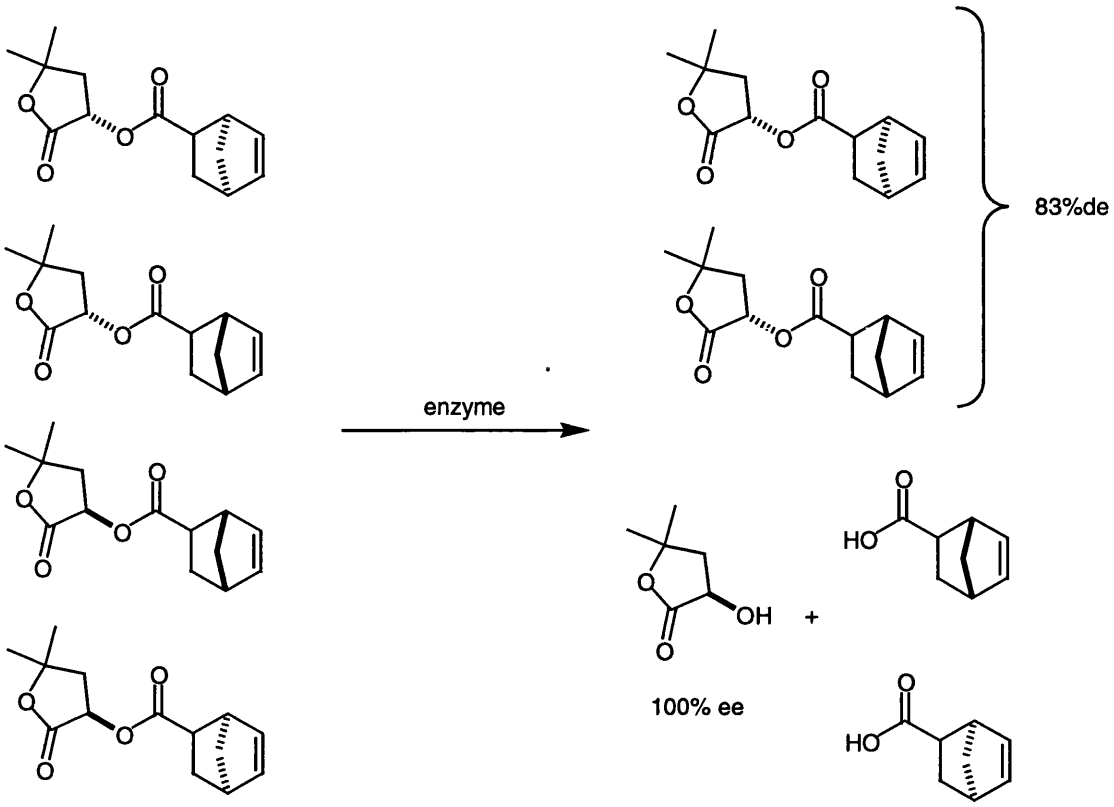


Figure 2: Shows four diasteroisomers of of the norbornene ester **41**.

Analysis of the norbornene ester **41** from the preparative TLC band showed an 83% ee of one of the remaining *endo* isomers (See appendix III for the Chiral GC chromatograph). As described in **Scheme 41**, the enzyme selectively hydrolysed the (*S*)- enantiomer of pantolactone but did not discriminate between the diastereoisomers of the norbornene portion of **41**, a very interesting result or effect the initial *exo*:*endo* ratio.

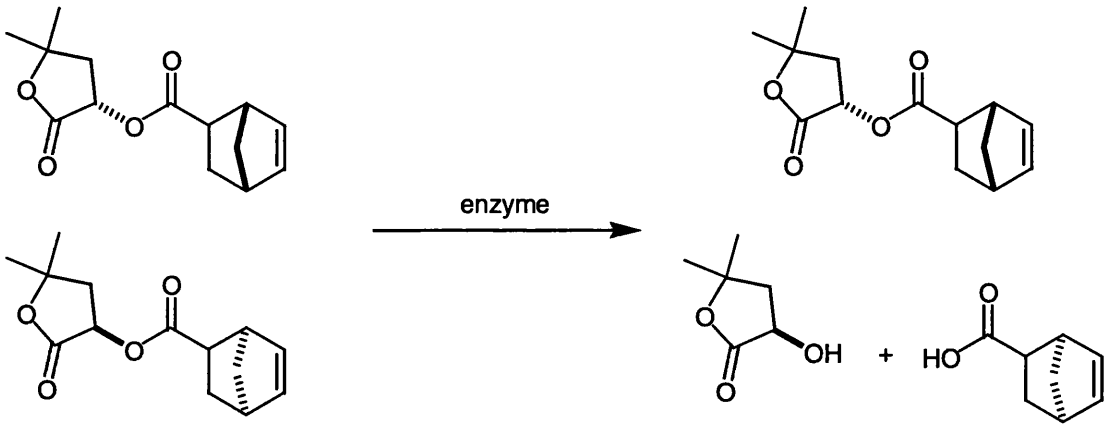


Scheme 41

Although we had succeeded in accomplishing what we had set out to do, we were intrigued by the role of aniline in the hydrolysis reaction and therefore investigated the reaction criteria further. An excess of aniline was found to be detrimental to the *kinetic resolution* as no reaction was observed. Solvents other than *t*BuOMe, namely methanol, hexane and ethyl acetate we also tried. Result showed that none of these solvents provided any success in the reaction what so ever; with or without aniline as an additive. We had found that the selective hydrolysis of the ester **41** seemed only to occur under a very specific set of reaction conditions. In general the reaction was optimal 25 °C with reactions at room temperature often failing or giving poor selectivities and conversions. It is also interesting to note that hydrolysis occurs at 40 °C but is non selective with racemic pantolactone being observed.

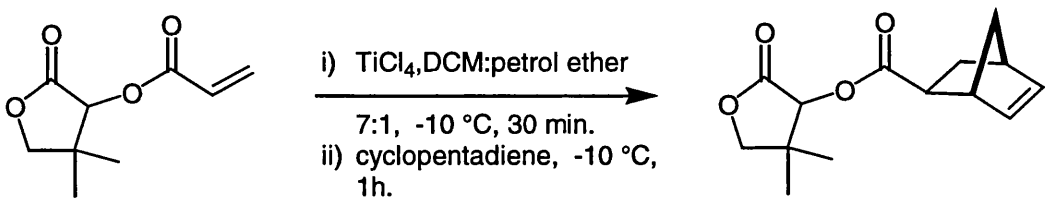
3.7 Enzymatic recognition of diastereoisomers

The resolution of diastereoisomers using an enzyme is not a new concept[21-23] but one we felt would be well investigated in light of our results of the hydrolysis of **41**. Our attentions turned to the resolution outlined below **Scheme 42**.



Scheme 42

To achieve the hydrolysis in **Scheme 42** we first had to synthesise the enantiomerically enriched norbornene ester starting material. This was facilitated using Helmchen's methodology.[10,24] We treated racemic pantolactone acrylate with cyclopentadiene and catalytic titanium chloride in THF at –78 °C which is described in **Scheme 43**. The substrate underwent an asymmetric Diels-Alder reaction yielding a mixture of diastereoisomers whih were racemic w.r.t. pantolactone and diastereomerically enriched w.r.t norbornene portion of the molecule.

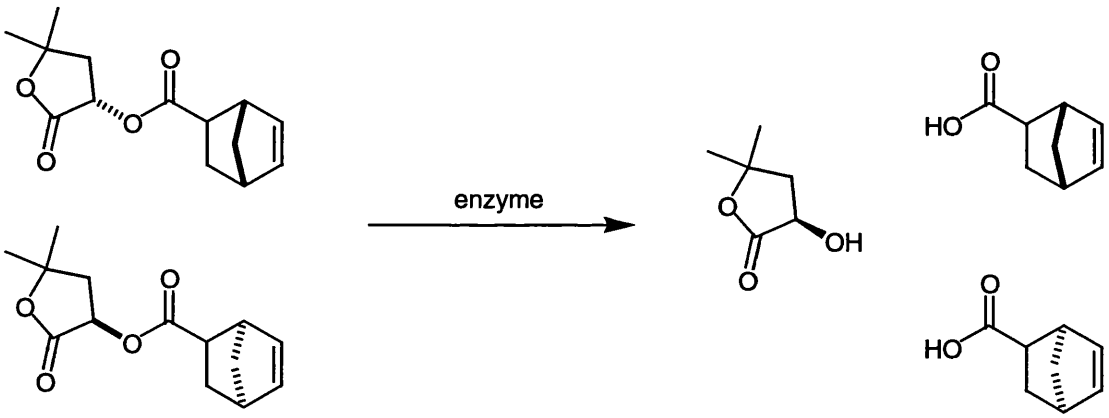


40

41
83% de
exo:endo 2:98

Scheme 43

We then planned to subject this mixture to enzymatic hydrolysis hoping that *Pseudomonas cepacia* lipase would selectively hydrolyse one enantiomer of pantolactone from the diastereomeric mixture. **Scheme 44** describes what would happen if the reaction was 100% selective, the best case scenario.

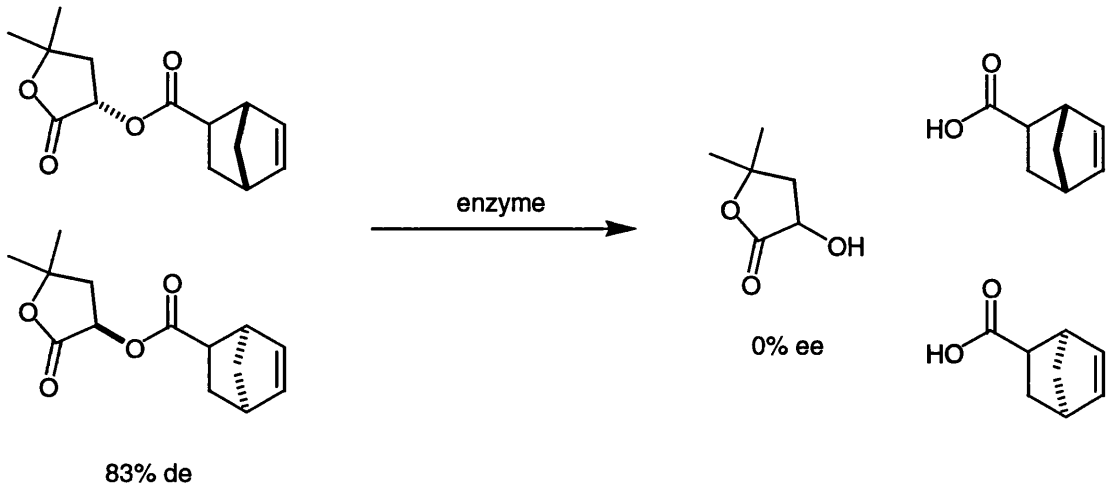


83% de

Scheme 44

In this instance norbornene carboxylic acid **61**, pantolactone **35** and the norbornene ester **41** would be formed enantiomerically pure. If this could be achieved it would be

a sophisticated piece of asymmetric synthesis. **Scheme 45** outlines the results from our preliminary hydrolysis studies.



Scheme 45

Our results were found to be disappointing as the enzyme hydrolysed pantolactone racemically. We also found that the ratio of diastereoisomers in the starting material had reduced from 83% de to 54% de w.r.t to the norbornene fragment. It seemed in this instance that the enzyme had hydrolysed the compound selectively in favour of the norbornene fragment of **41** and not w.r.t to pantolactone. Time did not allow further experiments.

3.8 Conclusion

The enzyme *Pseudomonas cepacia* lipase has been shown to selectively resolve pantolactone through esterification, transesterification and hydrolysis yielding enantiomerically enriched pantolactone, pantolacotne acetate and acrylate in good enatioselectivites. An extensive study into the *kinetic resolution* of pantolactone through its acrylate formation has been reviewed. Aspects of the reaction such as the

effect of solvent, temperature, acyl donor type and concentration are discussed. We can conclude from this that the *kinetic resolution* of pantolactone is more successful through acetate formation (99% ee) rather than acrylate (90% ee) formation.

Further to this similar studies have been carried out on Diels–Alder adducts of pantolactone acrylate using the enzyme *Pseudomonas cepacia* lipase with the aim of coupling Helmchen's methodology with enzymatic hydrolysis have been carried out with some success.

3.9 References

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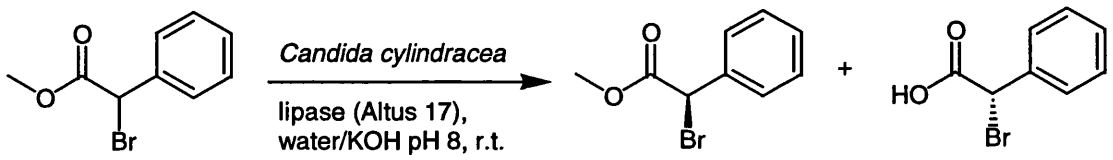
Section 4

The *Kinetic Resolution* of α-Chloro Acids

4.1 Introduction

This Chapter will describe work carried out towards the *kinetic resolution* of α-chloro esters. This work follows that of Jones et al[1,2] who recently reported the *kinetic resolution* of α-bromo-phenylacetic acid specifically using crosslinked enzyme crystals (CLECs) in his PhD thesis (The Williams group, Bath University). The authors found that CLEC-*Candida cylindracea* lipase (Altus-17) provided a fast reaction with good enantioselectivity as shown in **Scheme 46.**



62 **(*S*)-62** 80% ee 47% conv **(*R*)-63** 81% ee

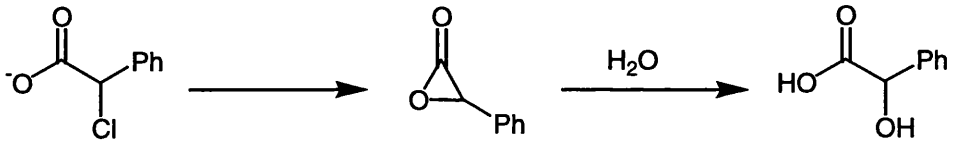
Scheme 46

In a short reaction time of 2.5h the (*R*)-enantiomer of phenyl acetic acid **(*R*)-63** was formed in 80% ee at 47% conversion of ester to acid. Conversely the enzyme *Pseudomonas cepacia* lipase was shown to hydrolyse the (*S*)-enantiomer of **(*S*)-62** in 65% ee at 32% conversion after 144 hours; a much slower reaction time and poor selectivity.

Although this is an excellent use of CLEC enzymes it is by no means the first example of the resolution of α-halo esters. Reviewing the literature of the *kinetic resolution* of α-fluorine,[3] bromine[4-6] and chlorine esters[7-9] it is clear that the enzyme *Candida cylindracea* lipase has been comprehensively used on a number of substrates.[4,6-10] Although substilin,[7] Pig liver esterase and *Pseudomonas fluorescens*

lipase[11] have also been reported. *Candida cylindracea* lipase has been reported to be used in both hydrolysis, transesterification and transamination reactions.

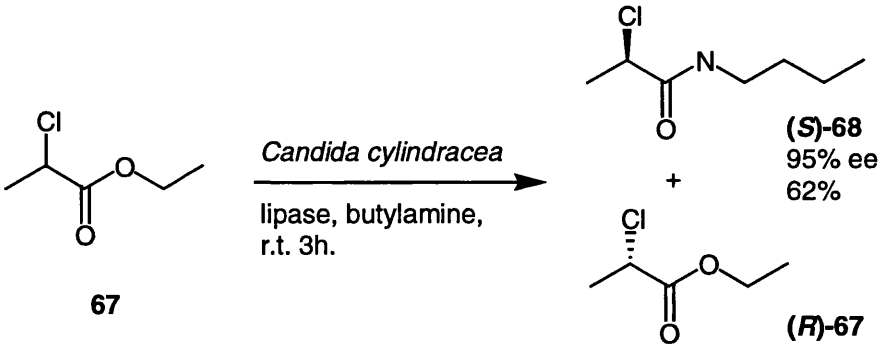
Although Jones et al. reported successful hydrolysis of α-bromo phenyl acetic acid **(*R*)-63** with *Candida cylindracea* lipase, there have been other less successful reports using *Candida cylindracea* lipase. Oxelbark[4] reported the formation of mandelic acid **66** from the enzymatic hydrolysis of the methyl ester of α-chlorophenyacetic acid in 0.1 M phosphate buffer at pH 6. It was found that, the mandelic acid **66** was formed in the last step by a non-enzymatic conversion of the carboxylate **64** to the acid **66** as shown in **Scheme 47**. Jones[2] did not report the formation of mandelic acid **66**.



64 **65** **66**

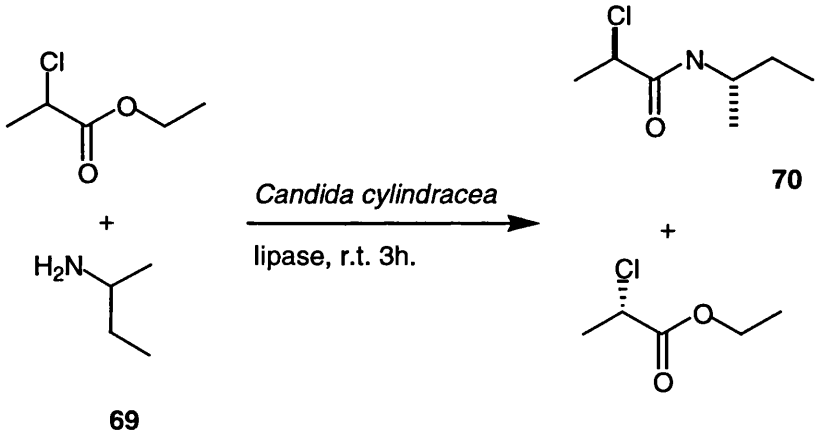
Scheme 47

As discussed previously (Section 1) Klibanov reported a very successful esterification of α-bromo propionic acid using butanol and the enzyme *Candida cylindracea* lipase.[6,12] Gotor has reported similar transamination experiments.[13] In these examples, α-chloro propionic acid methyl ester **67** was resolved with *Candida cylindracea* lipase and the primary amine butylamine as shown in **Scheme 48**. The racemic ester **67** was converted into its amide **(*S*)-68** in 95% ee and 62% conversion after 3h at room temperature.



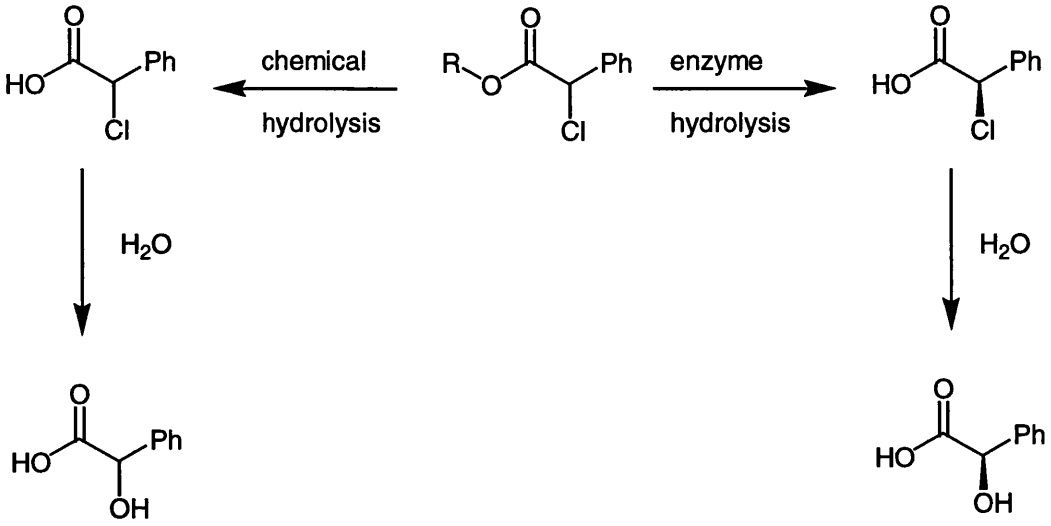
Scheme 48

Interestingly Gotor[14] repeated this work using a secondary amine and found that *Candida cylindracea* lipase resolved both ester and amine portions of molecule **67**. **Scheme 49** shows that the racemic α-chloro propionic ester **67** was resolved using 1-methyl propan-1-amine **69** forming the diasteroisomer **70**.



Scheme 49

We were prepared to observe more than just enzyme hydrolysis when working with α-halo esters. **Scheme 50** summarises the enzymatic and non-enzymatic reactions that may occur upon the *kinetic resolution* of α-chloro phenyl acetic acid **64**.

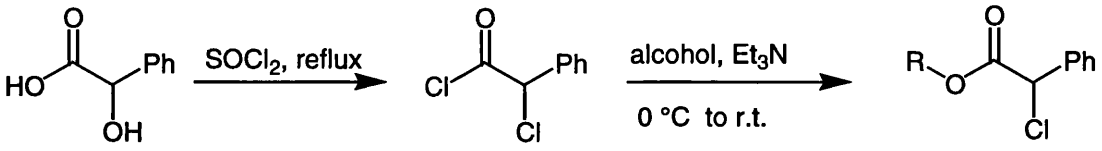


Scheme 50

The esters of α-chloro acids are reasonably labile at both the ester and α-carbon, therefore as well as ester hydrolysis, SN reactions can occur at the α-carbon. **Scheme 50** highlights that the ester is labile to both enzyme hydrolysis as well as being prone to chemical hydrolysis. The difference between the two processes being that the enzyme hydrolysis may be an enantioselective process where as the chemical hydrolysis will always occur non-selectively. It is therefore clear that chemical hydrolysis associated with selective enzyme hydrolysis has the possibility of decreasing the overall selectivity of the enzymatic resolution. Substituents at the α position may also be substituted by nucleophiles as shown in **Scheme 50**.

4.2 The synthesis of α-chloro acids and esters

Prior to any enzymatic resolutions being achieved, the esters **72**, **73**, **74**, **75**, and acid **64**, which are not commercially available, had to be synthesised. **Scheme 51** shows the synthesis of the esters. **72**, **73**, **74**, **75**, from mandelic acid **66**. Further more suitable HPLC methods were developed to enable the analysis the enantioselectivity of the enzymatic reactions, which were to follow.

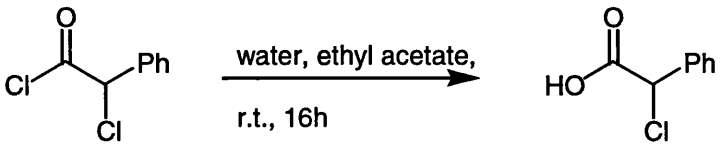


66 → **71** quantitative → R = Me, 95%, **72**; Et, 95%, **73**; CH_2CH_2Cl, 91% **74**; CH_2CF_3, 70% **75**

Scheme 51

Mandelic acid **66** was converted into the α-chloro acid chloride **71** using thionyl chloride. After distillation of the acid chloride (this can be purchased from Fluka) it was further reacted with 1 eq. of the relevant alcohol and triethylamine. Good yields were recorded for the methyl **72**, ethyl **73** and chloro ethyl **74** esters but trifluoro-ethyl ester **75** showed a poor yield. A longer reaction time may remedy this. Both enantiomers of the racemic esters **72**, **73**, **74** and **75** could be separated by chiral normal phase HPLC using an OD chiracel column (see appendix I).

Scheme 52 shows that the α-chlorophenyl acetic acid **64** can be synthesised by quenching the α-chloro acid chloride **71** with water.



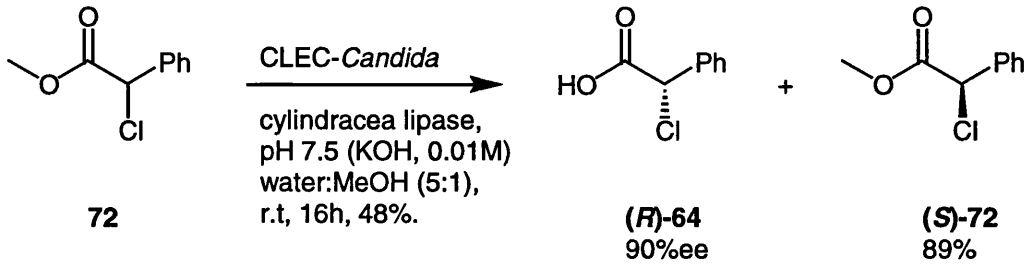
71 **64**
yield 52%

Scheme 52

The chiral separation of acid **64** by normal phase HPLC was more problematic than that of the ester separation. We eventually achieved resolution by adding 0.1% formic acid to the HPLC eluent (see appendix I). The acid is thought to protonate any basic sites on the silica column, which would otherwise impede the absorption of very polar compounds.

4.3 The *kinetic resolution* α-chloro acids

Studies into the *kinetic resolution* of the methyl ester of α-chloro phenyl acetic acid **64** were initially carried out using an autotitrator, which controlled the reactions pH to between 7 and 8 pH units, by the addition of potassium hydroxide. **Scheme 53** shows our preliminary reactions followed those of Jones et al, uing the crosslinked enzyme crystal of *Candida cylindracea* lipase, in water:MeOH mixtures of the ratio (5:1) at r.t. The reaction yielded a good resolution of the methyl ester **72** into the α-acid **64** in 90% ee and 48% conversion after 24h.



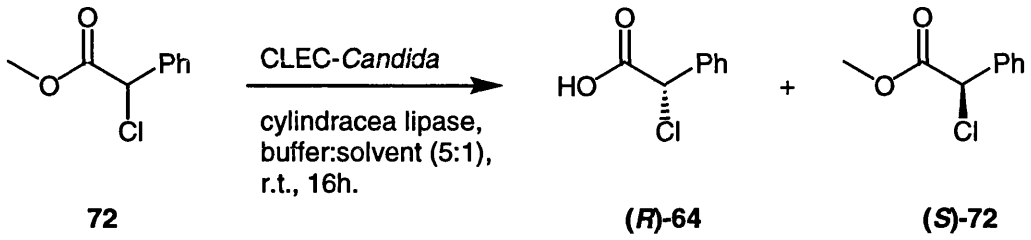
72 **(*R*)-64** 90%ee **(*S*)-72** 89%

Scheme 53

Satisfyingly, the enantioselectvity observed for the hydrolysis of **72** was higher than that of α–bromo phenyl acetic acid **(*R*)-64**. We rationalised that the increase in selectivity between α-Cl and α-Br substrates was due to the difference in atomic size of the halide. Chlorine is smaller than bromine and thus, there is a bigger difference in size between the phenyl group and halide in the α-chlorophenyl acetic acid ester n comparision witht the α-brormo ester and therefore, higher enzyme selectivities. The *kinetic resolution* of the α-chloro ester occurred in 16h, which was much slower than the 2-hour reaction time Jones reported for the hydrolysis of the α-bromo ester **62**.

Even though we had achieved a successful *kinetic* resolution of the methyl ester **72** to its corresponding acid **(*R*)-64** we wished to explore the *kinetic resolution* further. Firstly we examined the role of concentration. Our initial study shown in **Scheme 53** was conducted at 0.1g per 40 mL which is appreciable dilute. We set out to examine what would happen if we increased the concentration to 0.1g per 10 mL. We found at this new concentration our *kinetic resolution* took place giving 89% ee **(*R*)-64** at 48% conversion.

The *kinetic resolution* was also carried out in pH 7 phosphate buffer. **Scheme 54** shows that our initial studies using *Candida cylindracea* lipase and 5:1 buffer:solvent mixtures at room temperature gave some good results as highlighted in **Table 10.**



72 **(*R*)-64** **(*S*)-72**

Scheme 54

Solvent	Acid (ee%)	Ester (ee%)	Conversion (% by NMR)	E
EtOAc	90	49	25	30
MeOH	68	33	20	7.2
tBuOMe	72	19	10	17.4
Ether	82	19	11	12
Acetone	90	46	25	29
Cyclohexane	58	20	22	4.6
Acetonitrile	78	17	5	9.5
Toluene	80	10	9	9.9

Table 10: The *kinetic resolution* of the methyl ester of phenyl acetic acid using the enzyme *Candida cylindracea* lipase. 5% enzyme, 0.1g, Phosphate buffer:solvent 5:1, (5mL), r.t., 16h

The rate of reactions carried out in phosphate buffer were much slower than those in pH-controlled water but the enantioselectivities which ranged between 80-90% ee

were comparable with reactions carried out in pH controlled water. Solvents, which are particularly polar and non-miscible, showed poor results notably methanol, which gave a 68% ee of acid **(*R*)-64**. Non-polar, immiscible solvents such as cyclohexene also gave a poor enantioselectivity of 58% **(*R*)-64**. The *kinetic* resolution carried out in ethyl acetate gave an good selectivity of 90% ee in 25% conversion, which was comparable to our studies in pH-controlled water.

Using ethyl acetate or acetone as solvent the hydrolysis of **72, 73, 74** and **75** were tried as shown in **Table 11.**

Ester	Solvent	Acid (ee%)	Ester (ee%)	Conversion (% by NMR)	E
Me **72**	EtOAc	95	40	25	28
	Acetone	85	22	10	15
Et **73**	EtOAc	86	25	10	16
	Acetone	83	20	12	13
CH_2CH_2Cl **74**	EtOAc	88	34	28	21
	Acetone	84	84	45	30
CH_2CF_3 **75**	EtOAc	62	17	20	5
	Acetone	37	86	42	5.5

Table 11: A solvent study of the *kinetic resolution* of the esters **72** to **75** of phenyl acetic acid using the enzyme *Candida cylindracea* lipase. 5% enzyme, 0.025g, phosphate buffer:solvent, 5:1, (2.5mL), r.t., 16h.

The *kinetic resolution* of the methyl ester **72** in ethyl acetate showed the best selectivity of the study, with the ester being converted into phenyl acetic acid **(*R*)-64**

in 95% ee at 25% conversion of ester to acid. Our study showed better enantioselectivities could be achieved using the chloro-ethyl ester **77** over the corresponding ethyl ester **73** infact, the *kinetic resolution* of the chloro ethyl ester **74** in acetone gave the best E value. The activated trifluoroethyl ester **75** was formed in low enantioselectivity, which we attributed to tandem chemical hydrolysis.

Although, high enantioselectivities were achieved using phophate buffer, the conversions recorded for these reactions were poor as shown in **Table 11.** To improve the conversion of our reaction they were repeated at higher concentrations.

This strategy was successful giving excellent enantioselectivities for all three esters **72, 73** and **74** as shown in **Table 12.** The methyl ester **72** was converted in to its corresponding acid in 92% ee and 32% conversion. The ethyl ester **73** is converted into the acid **64** in 85% ee whilst the chlororthyl ester **74** is resolved in 90%ee. Notably the conversion of ethyl ester **73** to its corresponding acid occurred at a very slow rate in comparision to the ethyl and chloro-ethyl esters with 4% conversion of ester to acid being recorded after 16h as shown over in **Table 12.**

Ester	Acid (ee%)	Ester (ee%)	Conversion (% by NMR)	E
Me **72**	92	24	32	30
Et **73**	85	4	4	12
CH_2CH_2Cl **74**	90	93	45	64

Table 12: The effect of the ester group on the *kinetic resolution* of a group of phenyl acetic acid esters using the enzyme *Candida cylindracea* lipase. 5% enzyme, 0.1g, phosphate buffer:ethyl acetate, 5:1, (5mL), r.t. 16h.

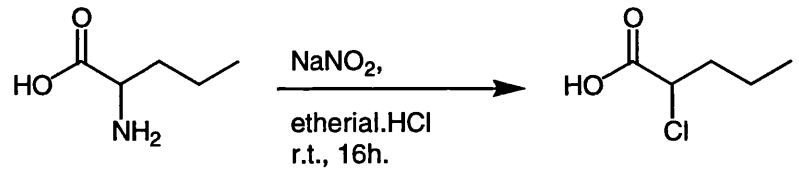
We repeated our *kinetic resolution* using *Pseudomonas cepacia* lipase-Altus 20, which was known to give the opposite selectivity in the hydrolysis of α-bromophenyl acetic acid **55**. As predicted, we observed the opposite selectivity for the ester **72**, **73** and **74** in our hydrolysis using Altus 20 although the selectivities were lower than observed when using *Candida cylindracea* lipase Altus 17 as seen in **Table 13**.

Ester	Enzyme	Acid (ee%)	Ester (ee%)	Conversion (% by NMR)	E
Me **72**	Altus17	91 (R)	89	47	63
	Altus 20	78 (S)	32	26	11
Et **73**	Altus17	86 (R)	62	35	24
	Altus 20	67 (S)	65	50	9.7
CH_2CH_2Cl **74**	Altus17	87 (R)	72	42	30
	Altus 20	39 (S)	22	30	2.8

Table 13: The *kinetic resolution* of a series of esters of phenyl acetic acid using the enzymes *Pseudomonas cepacia* lipase (Altus 20) and *Candida Cylindracea* lipase (Altus 17). 5% enzyme, 0.025g, phosphate buffer:solvent, 5:1, (2.5mL), r.t., 48h.

The methyl ester **72** was hydrolysed employing Altus 20 (*Pseudomonas cepacia* lipase) in to its corresponding acid in 78% ee at 47% conversion a lower selectivity than that observed with Altus 17 (*Candida cylindracea* lipase). Where as the ethyl ester **73** was hydrolysed giving its corresponding acid in 67% ee a much lower selectivity than those observed with Altus 17 but, with a better conversion than previously observed using Altus 17. A very poor selectivity was observed for the reaction of the chloro-ethyl ester that was converted into it corresponding acid in 22% ee.

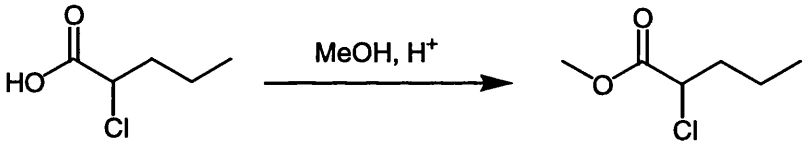
We now turned to the alkyl compound **79** to investigate if these types of compounds could also be resolved using our *kinetic resolution* protocol. **Scheme 55** shows that the synthesis of **78** was achieved by conversion of novaline **77** to α-chloro pentanoic acid **78**.



77 **78**

Scheme 55

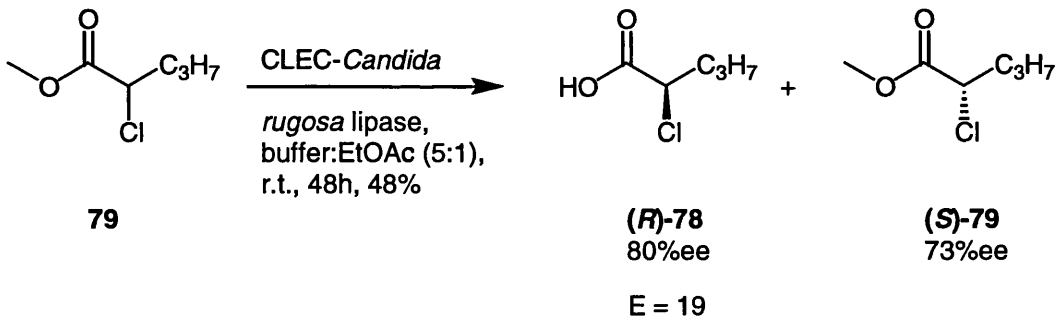
Diazotisation of norvaline **77** in ethereal hydrochloric acid gave the acid **78** in 94 % yield. Esterification could be achieved using standard esterification techniques employing methanol and catalytic amount of concentrated acid as shown in **Scheme 56**. This reaction proceeded in quantitative yield.



78 **79**

Scheme 56

Both racemic and enantiomerically pure **78** and **79** were synthesised in this way. The separation of compounds **78** and **79** could be achieved using chiral gas chromatography (see appendix II). **Scheme 57** shows the a successful *kinetic resolution* of the α–chloro ester **79** which was achieved using *Candida cylindracea* lipase yielding the acid **79** in 48% conversion and 80% ee. The enzyme *Pseudomonas cepacia* lipase was also tried but no reaction was observed.



Scheme 57

4.4 Conclusion

We have developed the *kinetic* resolution of both aryl and alkyl esters employing the enzymes *Candida cylindracea* lipase and *Pseudomonas cepacia* lipase. From these studies we have identified the best solvents and means of pH control for these *kinetic* resolutions. The activated ester **75** has been shown to be sensitive to chemical hydrolysis, which accompanied its enzymatic hydrolysis.

4.4 References

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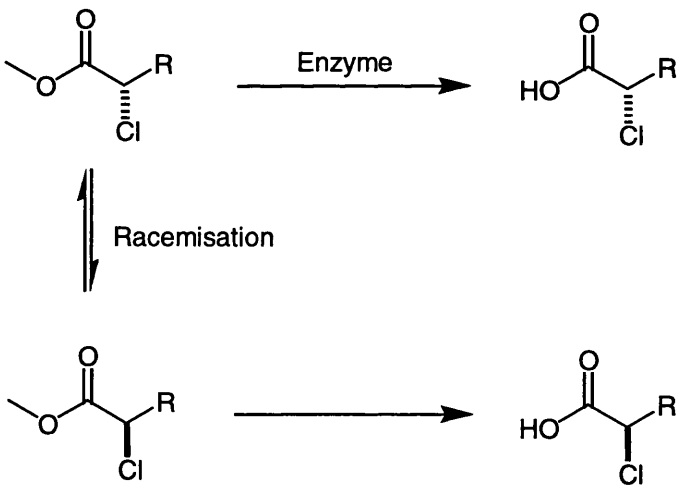
Section 5

The *Dynamic Kinetic Resolution* of α-Chloro Acids

5.1 Introduction

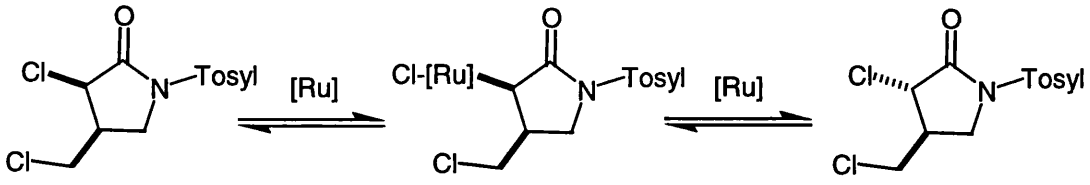
Chapter 4 outlined the hydrolysis of α-chloro esters using *Pseudomonas cepacia* lipase and *Candida cylindracea* lipase. In this chapter the selective racemisation of α-chloro esters vs. α-chloro acids is discussed along with the mechanism of racemisation and studies towards the *dynamic kinetic resolution* of α-chloro phenyl acetic acid.

Our strategy for the *dynamic kinetic resolution* of α-chloro esters is outlined below in **Scheme 58**. An α-chloro ester will be subjected to an enzymatic *kinetic resolution*, converting the ester into its corresponding carboxylic acid. In the presence of a chloride source an *in situ* racemisation would be carried out allowing the *dynamic* resolution of the α-chloro ester as shown in **Scheme 58**.



Scheme 58

Stereo-chemical scrambling of α-halo compounds has a literature precedent. **Scheme 59** shows how Slough[1] studied the racemisation of N-tosyl-2-pyrrolidinones using the ruthenium catalyst **80**.

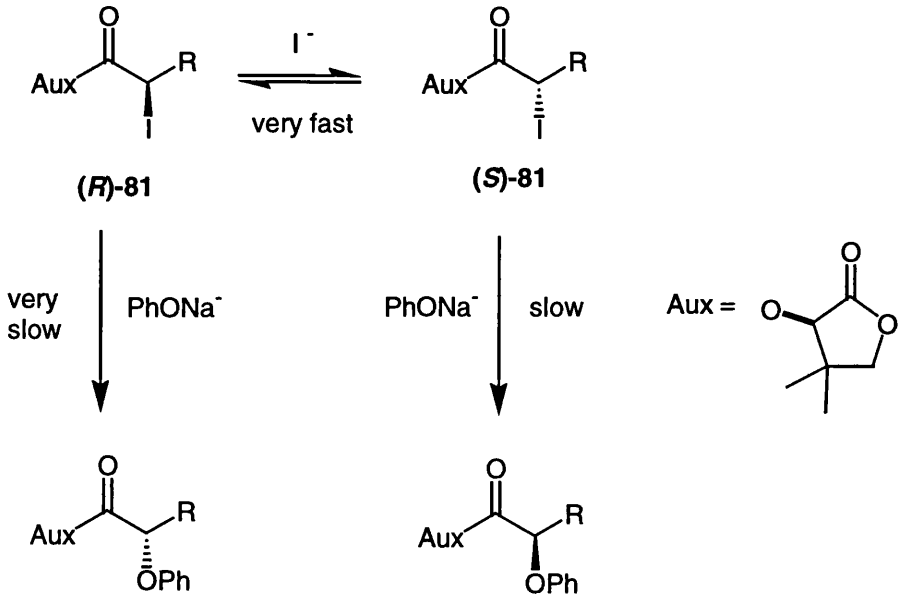


[Ru]=$(Ph_3P)RuCl_3$, **80**

Scheme 59

Slough demonstrated that racemisation occurred upon insertion of ruthenium into the C-Cl bond alpha to carbonyl group of the pyrrolidinone.

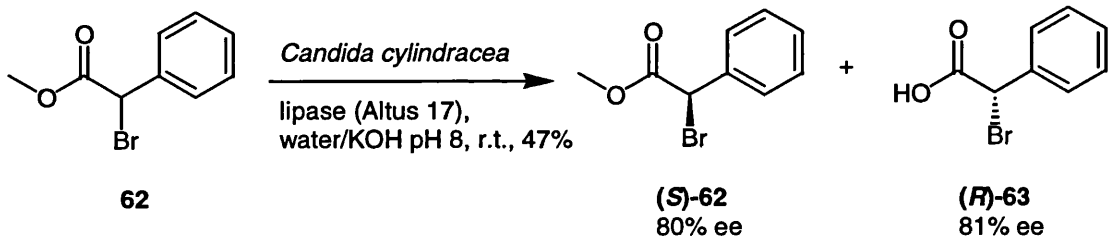
Koh and Durst[2] utilised a quaternary ammonium iodide salt to racemised the α-iodo carboxylic ester **81**. This racemisation protocol was used to synthesise a series of (*S*)-aryloxy acids as shown in **Scheme 60**.



Scheme 60

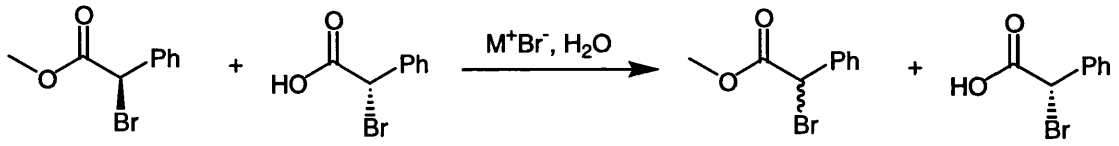
Ammonium iodide facilitates the racemisation of **81** enabling a constant supply of racemic material. The substitution of iodide with phenoxide was controlled by pantolactone chiral auxiliary, which favours the substitution of (*S*)-**81** rather than (*R*)-**81**. Caddick and Jenkins have also used this type of racemisation in the *dynamic kinetic* resolution of (*S*)- imino acid derivatives.[3]

Most recently, Jones has reported the *dynamic kinetic resolution* of α-bromo esters using *Candida rugosa* (*Candida cylindracea*) lipase. In this example an immobilised quaternary phosponium chloride (immobilised on brominated wang resin)[4] was used as a means of racemising the ester **62**.



Scheme 61

This methodology was developed after finding that the CLEC *Candida cylindracea* lipase could effectively resolve α-bromo-phenyl acetic acid **63** as shown in **Scheme 61**. Further to this Jones found that quaternary ammonium and phosphonium salts could effectively racemise the ester (*S*)-**62** but not the acid (*R*)-**63** at pH 7.0 as described in **Scheme 62** and **Table 14**.



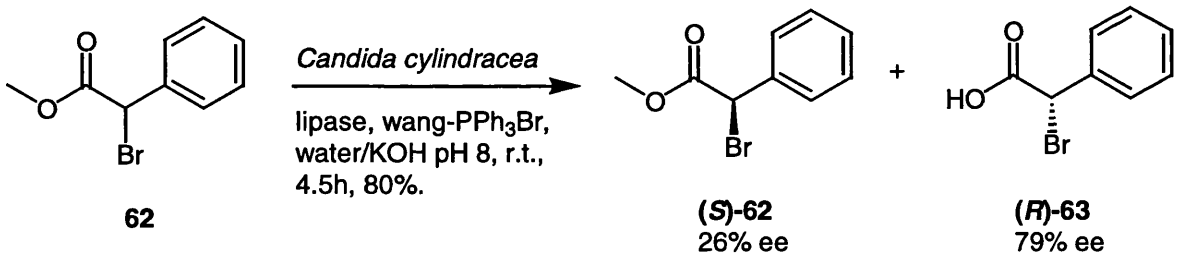
(*S*)-62 (*R*)-63 (*R/S*)-62 (*R*)-63

Scheme 62

Racemising Agent	Time	Initial		Final	
	h	Ester %ee	Acid %ee	Ester %ee	Acid %ee
Bu_4PBr	18	81	33	4	31
$C_{16}H_{21}PPh_3Br$	6	55	38	5	36
$BnPPh_3Br$	2	82	74	40	69
$(C_8H_{17})_4NBr$	72	30	56	6	52
Wang-PPh_3Br	2	43	61	1	58

Table 14: Racemisation of the acid **(*S*)-63** and the ester **(*R*)-62** at pH 7 in water:MeOH (5:1).

Combination of the enzymatic hydrolysis of the ester **62** and the racemisation protocol using the enzyme *Candida cylindracea* lipase and racemising agent tetraphenyl phosphonium bromide gave 8% ee of the acid **(*R*)-63** at 70% conversion. Jones et al concluded that the enzyme and racemising agent were not compatible. Immobilisation of the racemisation agent onto brominated wang resin overcame these problems giving the acid **(*R*)-63** in 79% ee in 4.5h at room temperature with the remaining ester having 26%ee[5] as shown in **Scheme 63**. This clearly shows a *dynamic kinetic resolution* of the α-bromo ester **62** to its corresponding acid **(*R*)-63**.

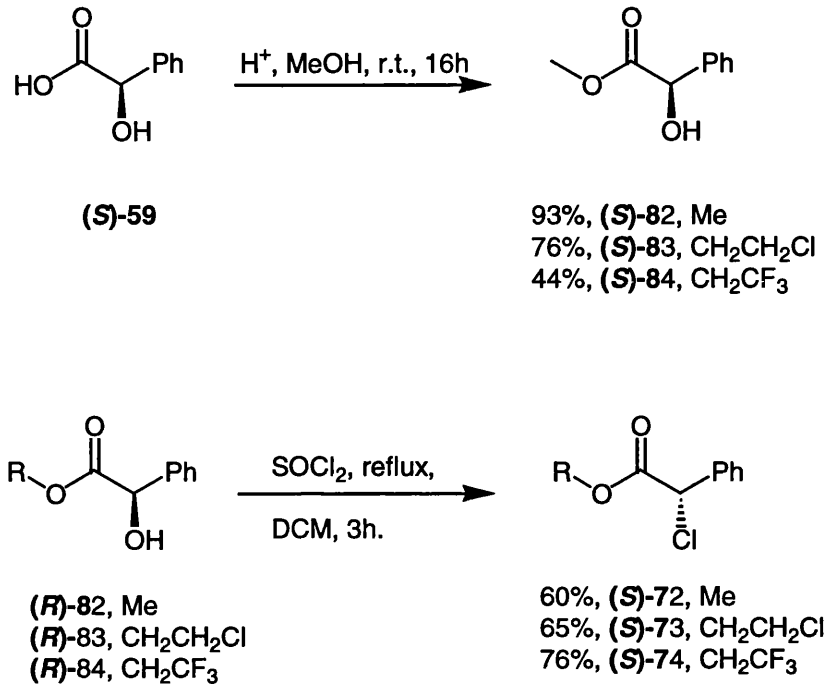


Scheme 63

We wanted to try to repeat the racemisation and *dynamic kinetic resolution* of α-bromo acids using α-chloro substrates. We were unsure that the direct application of Jones racemising methodology would be applicable to the racemisation of α-chloro esters. We had rationalised from empirical rule that Cl^- is a poorer nucleophile than Br^- and therefore it would be more difficult to racemised the α-chloro ester in relation to an α-bromo ester.

5.2 The synthesis of enantiomerically enriched α-chloro esters and acids.

To enable us to carry out racemisation studies to take place, chiral α-chloro acid **(*R*)-64** and α-chloro ester **(*S*)-72** were prepared. The synthesis of enantiomerically enriched α-chloro ester was achieved using chiral mandelic acid **(*S*)-66** as a starting material. Esterification of mandelic acid using the relevant alcohol and concentrated hydrochloric acid was carried out as shown in **Scheme 64**.

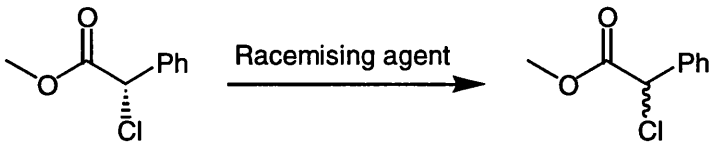


Scheme 64

The α-hydroxy group was substituted using thionyl chloride giving **(*R*)-72**, **(*R*)-73**, **(*R*)-74**. During this step some racemisation of the esters occurred.

5.3 Racemisation studies

Our initial racemisation studies examined the racemisation of the methyl ester of phenyl acetic acid **(*R*)-72** with a variety of ammonium and phosphonium chlorides along with triethyl amine hydrochloride as shown in **Scheme 65** and our results recorded in **Table 15**.



(*R*)-72 **(*R*/S)-72**

Scheme 65

Racemisation Agent	Final ee% of the ester
$Et_3N.HCl$	44
Adogen 464 (Methyl(trialkyl(C_8-C_{10})) ammonium chloride)	22
Aliquat 336 (Tricaprylmethyl ammonium chloride)	38
Bu_4PCl	44
Bu_4NCl	44
Me_4PCl	44
Ph_4PCl	44
$Me(Ph)_3PCl$	44
$(heptyl)_4AsCl.H_2O$	44

Table 15: The racemisation of the ester **(*R*)-72** (initial ee% 44) using a variety of racemising agents in pH 7 water:MeOH (5:1). Racemising agent (0.6 eq), 25 °C, 24h, 20 mg in 1 mL.

Our results showed that only the long chain alkyl ammonim chlorides such as Adogen 464 and Aliquat 336 were found to racemised the ester **(*R*)-72** at room temperature. As we had expected the racemisation of **(*R*)-72** was slower than that of the methyl ester of α-bromo phenyl acetic acid **(*S*)-63**. These differences can be explained by considering the differences between Br^- and Cl^- as both nucleophiles and leaving groups. Bromide ion is a better nucleophile and leaving group than chloride because

a bromide ions charge is more delocalised than chloride due to its larger atomic radius.

After finding that the racemisation of **(*R*)-72** was slow at room temperature we began to optimise the reaction and increase the rate of racemisation. Firstly, we increased the temperature of our racemisation studies from rt. to 40 °C, which we found to increase the rate of racemisation such that **(*R*)-72** (initial 44 ee%) completely racemised in 24h. The same result was found when we reduced the reaction time to 8h. Increasing the temperature by only 20 °C gave a great improvement to the rate of racemisation. Keeping the temperature at 40 °C we tried to optimise the reaction by changing the reaction parameters one by one starting with solvent type. We knew Jones had only reported the use of methanol/water mixtures in his racemisation experiments. Therefore, we carried out a series of experiments to find out how would other solvent mixtures fair? **Figure 3**.

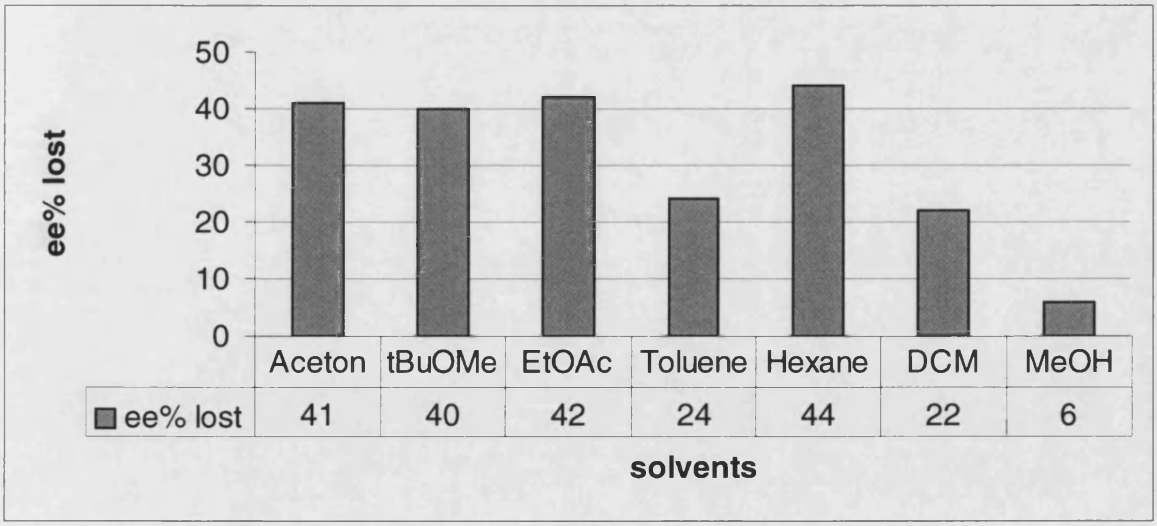


Figure 3 Racemising experiments investigating the role of solvent. Adogen, 40 °C, 6h, 20 mg in 1 mL.

Ethers, ketones and hydrocarbons were shown to be the best solvents to promote the racemisation of **(*R*)-72** whereas methanol was the worst. We rationalised that methanol slowed the rate of racemisation down because it could readily solvate the Cl^- nucleophile and thus impede its action.

Upon trying to complete the *dynamic kinetic* resolution of **(*R*)-72** we wished to use this racemisation protocol in parallel with enzymatic hydrolysis therefore water must be present as a solvent. Racemisation studies in water solvent mixtures of the ratio 5:1 water:solvent were then studied as shown in **Figure 4**.

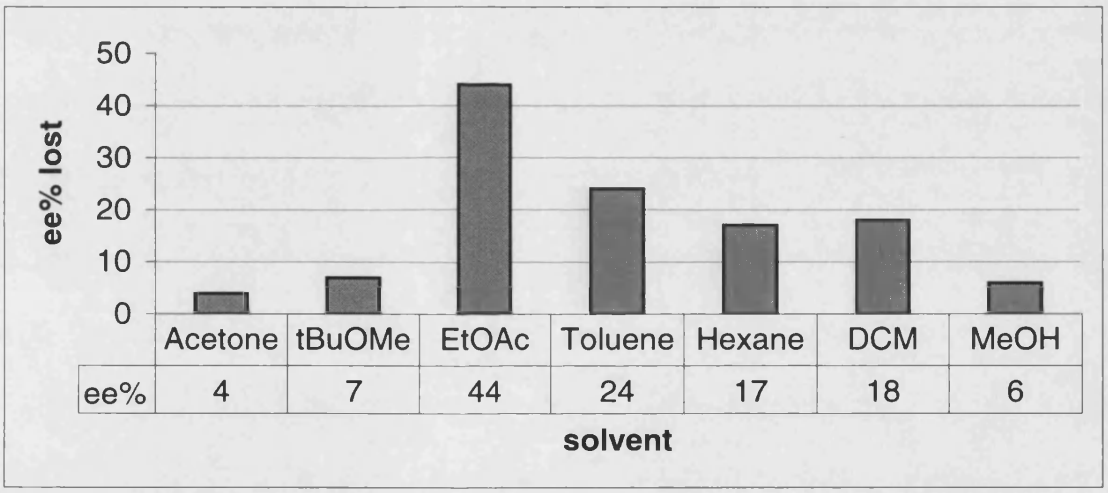


Figure 4 Racemising experiments investigating the role of solvent. Adogen (0.5 eq), 40 °C, 6h, 20 mg in 1 mL, (5:1, water:solvent).

Our results showed that the rate of racemisation was slower in water:solvent mixtures than that in anhydrous solvents. Again we theorised that the water like methanol would solvate the Cl^- and therefore impede the rate of racemisation. The results also showed that the rate of racemisation was slower in miscible solvents such as methanol or acetone and slightly faster in immiscible solvent such as hexane or dichloromethane. Again we attributed this to the ease of solvation of the nucleophile.

We also noted that there was no direct trend in polarity vs. rate of racemisation. For, example dichloromethane a polar solvent showed a similar rate of racemisation to hexane a non-polar solvent whereas, tBuOMe a solvent, which is similar in polarity to EtOAc, showed a very different rate of racemisation.

The role Lewis acids could play in the rate of racemisation of **(*R*)-72** were investigated. A variety of Lewis acids were added to standard racemisation reactions at 10 mol% concentration and were carried out alongside a blank reaction which had no Lewis acid present, the results of our study are recorded in **Figure 5**.

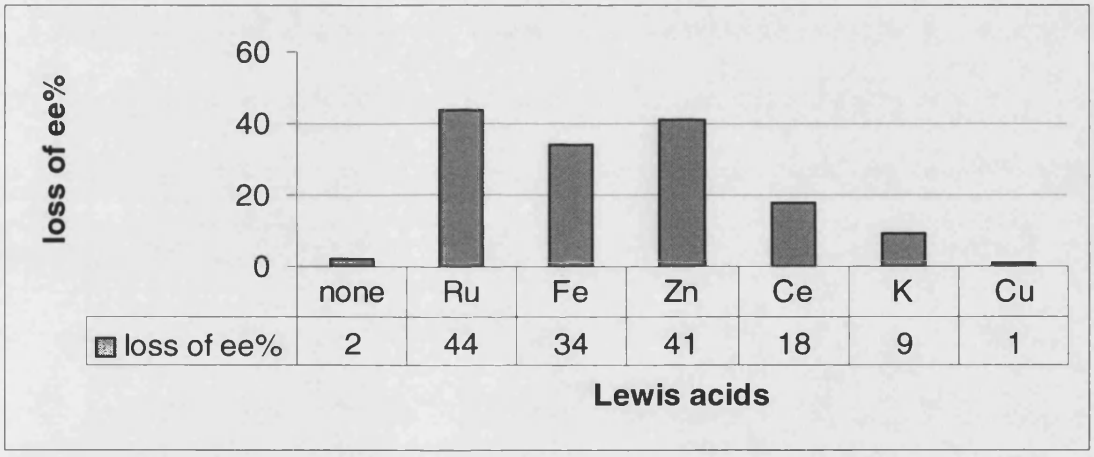


Figure 5: Racemisation experiment investigating the role of lewis acids on the rate of racemisation. Adogen 0.6eq, 40 °C, 20mg in 1 mL, 75 mins. All Lewis acids were employed as their chloride salts.

Ruthenium chloride, iron chloride and zinc chloride catalysed the racemisation of **(*R*)-72** using 0.6 equivalents of adogen. Interestingly, potassium chloride not typically known for it Lewis acid ability had some effect on the rate of racemisation relative to the blank experiment.

Now that we had optimised the racemisation conditions for **(*R*)-72, Table 16** shows the results we recorded upon repeating our initial racemisation study using our new optimised conditions.

Racemising agent	Ee% loss	Racemising agent	Ee% loss
$Et_3N.HCl$	0	Bu_4NCl	7
Aliquat	42	Me_4PCl	4
Adogen	41	Ph_4PCl	4
Bu_4PCl	7	$MePh_3PCl$	22

Table 16: The racemisation of **(*R*)-72** under optimal conditions. Adogen 0.6eq, 40 °C, 20mg in 1 mL (5:1 buffer:ethyl acetate, 6 h.

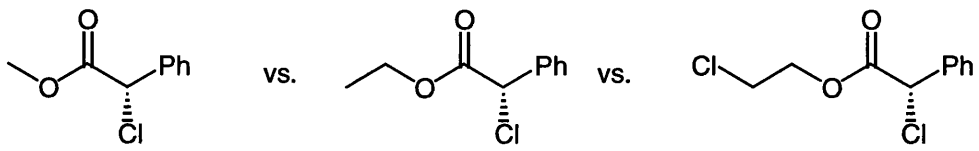
The racemisation experiments summarised in **Table 16** show a five times increase in the rate of racemisation from initial studies following the work of Jones et al described previously in **Table 14** to our new optimal conditions.

With our racemisation protocol optimised we wondered if we could reduce the amount of racemising agent and add a Lewis acid to compensate for the loss in rate. This was done because we knew that there had been problems combining racemising agent and enzyme in the *dynamic kinetic resolution* studies of α-bromo phenyl acetic acid our results are reported in **Table 17**.

Racemising agent	Lewis acid	Ee% loss
Adogen (10 mol%)	None	12%
Adogen (10 mol%)	$ZnCl_2$ (10 mol%)	24%

Table 17: The racemisation of (*R*)-**72** reducing the amount of racemising agent below 0.5 eq. 40 °C, 20mg in 1 mL, 6 h

We found that decreasing the amount of racemisation agent used did decrease the rate of racemisation with only 12% ee being lost in 16h. Equally decreasing the amount of racemising agent but adding a Lewis acid catalyst gave an enhanced rate, in this instance we recorded a loss of 24% ee in 16h. In fact the rate of racemisation doubled when a Lewis acid was used but wasn't comparable to a parallel experiment using 0.6 eq of adogen where 41%ee was lost in the same time (see table 16). Now that we had comprehensively reviewed the racemisation condition for (*R*)-**72**, we were intrigued to find out how the rate of racemisation would be affected by substrate type. We were specifically interested in varying the ester portion of the molecule and the 'R' group. By combining equal amounts of (*R*)-**72**, (*R*)-**73**, (*R*)-**74**, in one reaction. Our results showed that the more activated the ester the faster the racemisation as recorded in **Table 18.**

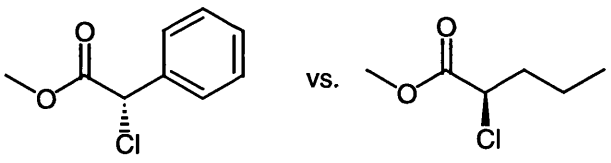


(*R*)-**72** (*R*)-**73** (*R*)-**74**

Ester	Ee% lost
Me **72**	7
Et **73**	15
CH_2CH_2Cl **74**	27

Table 18: The comparison of ester type upon the rate of racemisation. Adogen 0.6eq, 40 °C, 20mg in 1 mL, 1h.

The role of 'R' group was investigatd by comparing the reaction of (*R*)-**72** with that of the enantiomerically pure methyl ester of α-chloro propionic acid (*S*)-**79**. The results from this experiment show that substituting an aryl group for an alkyl group resulted in grinding the rate racemisation to a stand still as shown in **Table 19**. Unfortunately this then limited the types of substrates, which could be racemised in this way therefore, reducing the scope of our racemisation methodology.

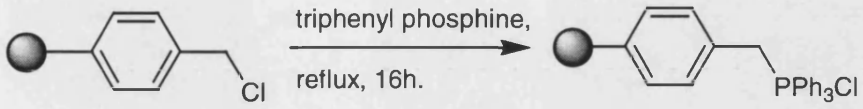


(*R*)-72 (*R*)-79

R group	Ee% lost
Ph	44
Propyl	0

Table 19: The comparison of 'R' group upon the rate of racemisation Adogen 0.6eq, 40 °C, 20mg in 1 mL, 16h.

The rate of racemisation of ester (***R***)-**72** was investigated using the immobilised phenyl phosphonium chloride **86**. The phosphonium salt was prepared by the addition of chlorinated Merrifield resin **85** in toluene and treating it with ten equivalents of triphenyl phosphine under reflux for 16h as shown in **Scheme 66**.



85 **86**

Scheme 66

The extent of formation of **86** was measured gravimetrically by weighing the recovered triphenyl phosphine. Racemisation experiments comparing our immobilised Cl^- source **86** with adogen and aliquat showed that in water solvent mixtures controlled at pH7 the resin racemised (***R***)-**72** more slowly than adogen or aliquat and performed better in hexane than in ethyl acetate **Table 20**.

Racemising agent	Equivalents	$ZnCl_2$	Solvent	Loss of ee%
Adogen	0.5	-	EtOAc	36
	0.1	0.1 eq	Hexane	30
Aliquat	0.5	-	EtOAc	44
	0.1	0.1 eq	Hexane	21
Resin	0.5	-	EtOAc	8
	0.1	0.1 eq	Hexane	16

Table 20: Racemisation of the ester (***R***)-**72** with different sources of chloride. 0.25ml in 1ml, 8h.

Enzyme reactions are not always carried out in water solvent mixture but often carried out in phosphate buffers. Therefore we wondered if our racemisation protocol would work in phosphate buffer, our results are shown in **Table 21.**

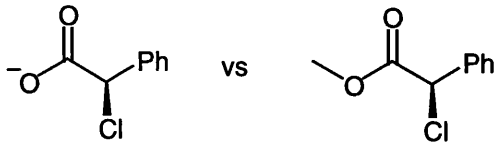
Temp °C	Racemising agent	$ZnCl_2$ (eq)	Loss of ee%
25	Adogen 0.5 eq	-	14
25	Adogen 0.5 eq	0.1	27
25	Resin 0.5 eq	-	33
25	Resin 0.1 eq	0.1	33
40	Adogen 0.5 eq	-	25
40	Adogen 0.5 eq	0.1	24
40	Resin 0.5 eq	-	44
	Resin 0.1 eq	0.1	41

Table 21: Racemisation of the ester **(*R*)-72** with different sources of chloride. 0.25g in 1ml, 16h.

The Merrifield resin based racemisation agent worked well at both 25°C and 40 °C. In comparison with the experiment carried out in water:solvent mixtures the rates of racemisation in phosphate buffer was significantly slower.

5.4 Acid vs. ester racemisation

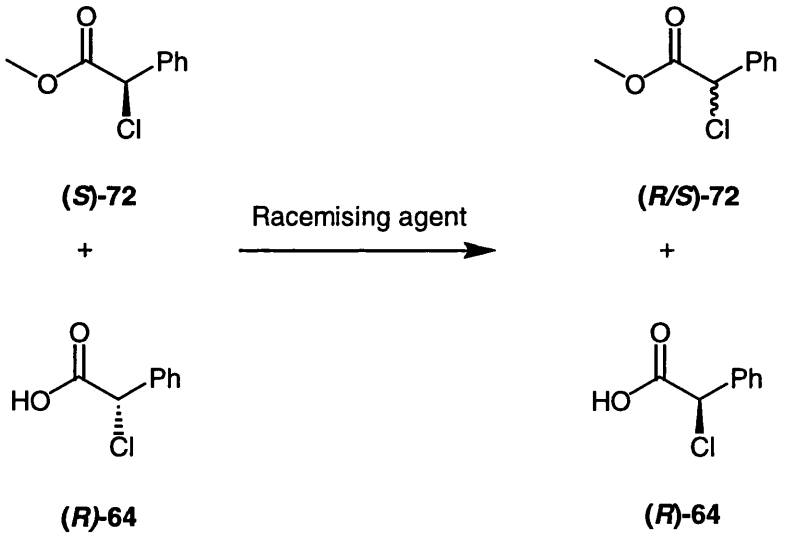
To enable our *dynamic kinetic* resolution to succeed we needed to suppress the racemisation of the α-chloro acid **(*S*)-64**. We planned to suppress the racemisation of the acid by controlling the pH between 7 and 8 as shown in **Figure 6.**



(*S*)-64 **(*S*)-72**

Figure 6: Acid vs. ester racemisation.

We proposed that the racemisation of the ester would proceed by SN2 displacement of the Cl^- ion. In this instance the chloride ion would travel towards the δ^+ of the C-Cl bond forming a tetrahedral intermediate which would be stabilised by donation of electron density to the π^* orbitals of the carbonyl functionality. This isn't true in the case of the carboxylate because in this instance the π^* orbital is already filled through resonance of the carboxylate disabling the carboxylate racemisation.



Scheme 67

Our acid vs. ester racemisation studies were planned to incorporate the reactions conditions found to be important in our previous ester racemisation studies, for example, solvent, pH control and temperature as shown in **Scheme 67**.

The results in **Table 22** show that in pH 7-8 controlled water:ethyl acetate mixtures the acid **(*R*)-64** racemisation could be slowed down but not entirely stopped.

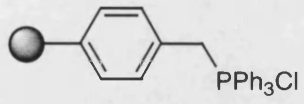
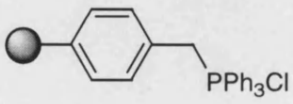
Racemising Agent	**Initial ee%**		**Final ee%**	
	Ester	**Acid**	**Ester**	**Acid**
Aliquat 336 (Tricaprylmethyl ammonium chloride)	31	64	0	42
Adogen 464 (Methyl(trialkyl(C_8-C_{10})) ammonium chloride)	31	64	0	41
Bu_4NCl	31	64	20	61
Bu_4PCl	31	64	11	55
$MePh_3PCl$	31	64	22	36
Resin	31	64	0	53
 **86**				
Resin (24h old)	31	64	0	17
 **86**				

Table 22: Acid vs. ester acemisation of the ester **(*S*)-72** and acid **(*R*)-64** with different sources of chloride at pH 7-8, Adogen 0.6eq, 40 °C, 20mg in 1 mL, 16 h

The butylammonium chloride and butylphosphonium chlorides seemed to be the best reagents for the racemisation of the ester **(*S*)-72** but not acid **(*R*)-64**. On the other hand Adogen and Aliquat were shown to be excellent racemisation agent for ester but didn't control acid racemisation as well as tetrabutylammonium and phosphonium chlorides. We also observed a demise in the effectiveness of the resin **86** over time. Resin used 24 hours after synthesis proved detrimental to preserving the acid **(*R*)-64** enantioselectivity.

Repeating acid vs. ester racemisation experiments in phosphate buffer gave disappointing results. We found that the acid racemisation could not be controlled effectively in phosphate buffer, our results are shown in **Table 23**.

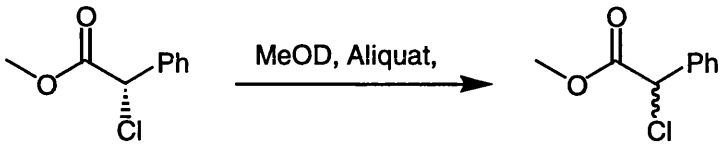
Racemising	**Initial ee%**		**Final ee%**	
Agent	**Ester**	**Acid**	**Ester**	**Acid**
Bu_4PCl (1 eq)	44	95	21	34
Bu_4PCl (1 eq), ZnCl (10 mol%)	44	85	15	45

Table 23: Acid vs. ester acemisation of the ester **(*S*)-72** and acid **(*R*)-64** with different sources of chloride at pH 7-8, Adogen 0.6eq, 40 °C, 20mg in 1 mL, 16 h.

5.5 Investigations into the mechanism of racemisation

Although Jones et al proposed that the mechanism of racemisation was SN2 we knew that without further experimental data we could not be sure of that. Although we did have some evidence that the mechanism is SN2 from solvent studies.

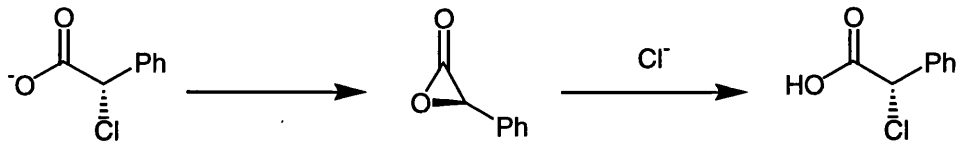
It was possible that the mechanism of racemisation may occur via enolisation but this was ruled out by carrying out a racemisation experiment in the presence of deuterated methanol as shown as **Scheme 68.**



(*R*)-72 **(*R*/S)-72**

Scheme 68

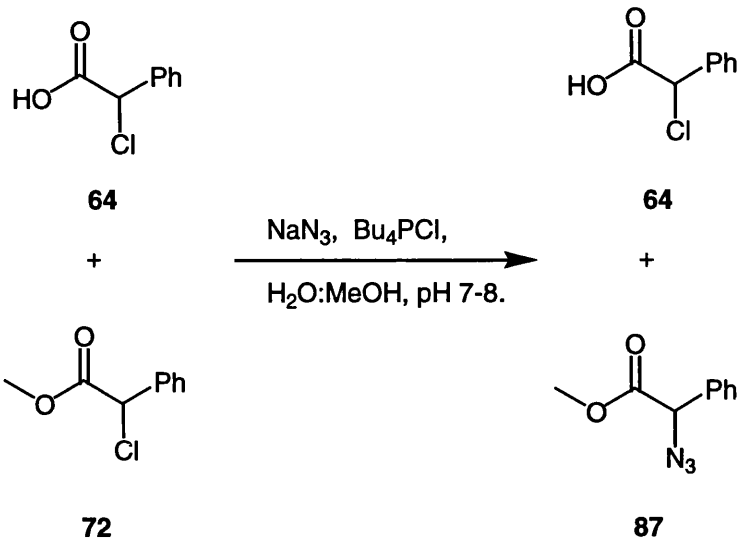
We observed no deuterium incorporation and so ruled out enolisation as a mechanism for racemisation. We had also proposed that at pH 7 the ester **(*R*)-72** racemised but acid **(*R*)-64** was present as it carboxylate and did not react in anyway. A contradictory theory is that the carboxylate does infact racemised but goes through a double inversion as shown in **Scheme 69** and therefore doesn't seem to racemise.



(*R*)-64 **(*S*)-65** **(*R*)-64**

Scheme 69

We tested this theory by adding sodium azide to an acid vs. ester racemisation experiment controlled at pH7-8 as shown in **Scheme 70.**



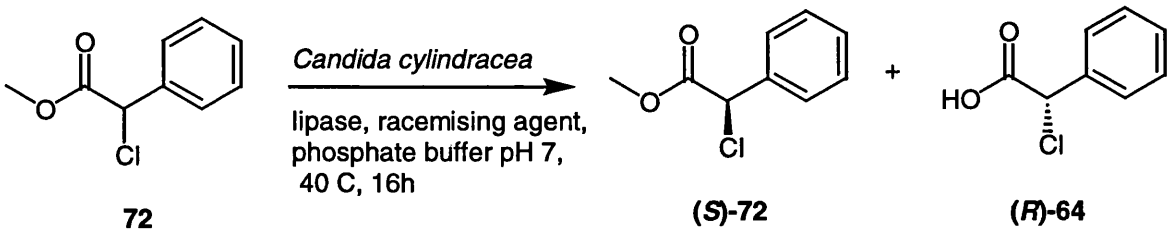
Scheme 70

Proton NMR analysis showed azide formation in the ester **87** but not in the acid which suggested that the acid does not form α-lactones but remains as its carboxylate.

5.6 Studies towards the *dynamic kinetic resolution* of α–chloro esters.

With the racemisation and enzymatic *kinetic resolution* of α-chloro phenyl acetic acid **72** completed a combination of those protocols was expected to provide the *dynamic kinetic resolution* (DKR) of α-chlorophenyl acetic acid **64**.

We knew from our acid vs. ester racemisation studies that we had to control the pH of our DKR reaction using an auto-titrator and not phosphate buffer to inhibit the acids racemisation. We also knew that activated esters had been found to be unstable in pH controlled water and therefore wouldn't be suitable for our DKR studies. The methyl ester **72** was chosen as the substrate we would use in our DKR studies, which were to be conducted in pH controlled water. Our preliminary reactions are outlined in **Scheme 71**, and results illustrated in **Table 24.**



72 (*S*)-72 (*R*)-64

Scheme 71

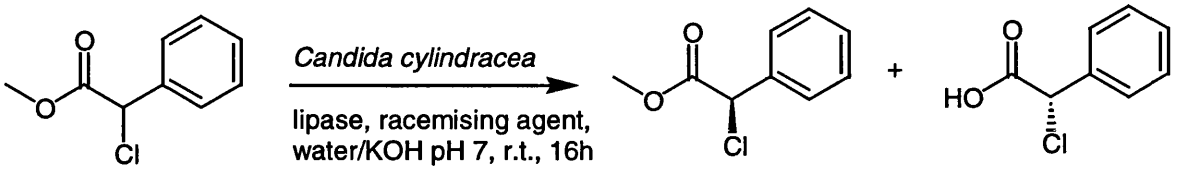
Racemisation agent (eq)	Lewis acid	Ester	Acid	Conversion
Adogen 0.6	None	0	26	33
Adogen 0.6	$ZnCl_2$ 10 mol%	35	0	42
Adogen 0.1	$ZnCl_2$ 10 mol%	62	0	45

Table 24: *Dynamic kinetic resolutions* carried out in phosphate buffer. 40 °C, 20mg in 1 mL, 16 h

As expected, controlling the pH using phosphate buffer didn't completely prevent acid (*R*)-**64** racemisation. Equally, the enantioselectivities of our *dynamic kinetic resolutions* carried out in phosphate buffer were poor with 33% ee being recorded when 0.6 eq of adogen was used. An increase in selectivity was observed when the concentration of adogen was decreased from 0.6 eq to 0.1 eq and $ZnCl_2$ used to compensate for the loss racemisation rate. This certainly, indicated that there was an incompatibility between the enzyme and the racemisation agent.

Dynamic kinetic resolution experiments were also carried out on an auto-titrator using either Bu_4PCl at 0.6 eq or 0.1eq Bu_4PCl and 0.1eq zinc chloride. These experiments yielded reasonable *dynamic kinetic resolutions*, although the enantioselectivity of the

processes were still compromised in comparison to the *kinetic resolution* carried out under the same conditions as shown in **Scheme 72**, and results reported in **Table 25.**



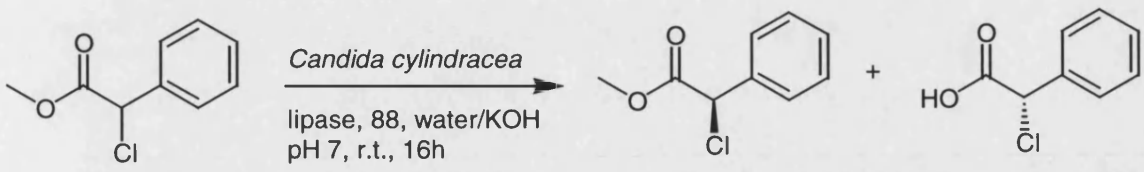
72 **(*S*)-72** **(*R*)-64**

Scheme 72

Racemisation agent (eq)	Lewis acid	Ester (ee%)	Acid (ee%)	Conversion (%)
Bu_4PCl 0.6	None	37	62	59
Bu_4PCl 0.1	$ZnCl_2$ 10 mol%	21	62	61

Table 25: *Dynamic kinetic resolutions* carried out in pH 7-8 controlled water. 40 °C, 20mg in 1 mL, 16 h

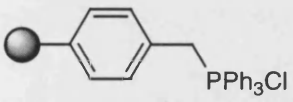
It was obvious that the DKR would work best in pH controlled water and that there were issues concerning compatibility between enzyme and racemisation agent. Therefore we employed the immobilised racemisation agent **86** as shown in **Scheme 73**, and our results recorded in **Table 26**.



72 **(*S*)-72** **(*R*)-64**

Scheme 73

Racemisation agent (eq)	Lewis acid	Ester (ee%)	Acid (ee%)	Conversion (%)
Resin 0.6eq	None	22	90	90



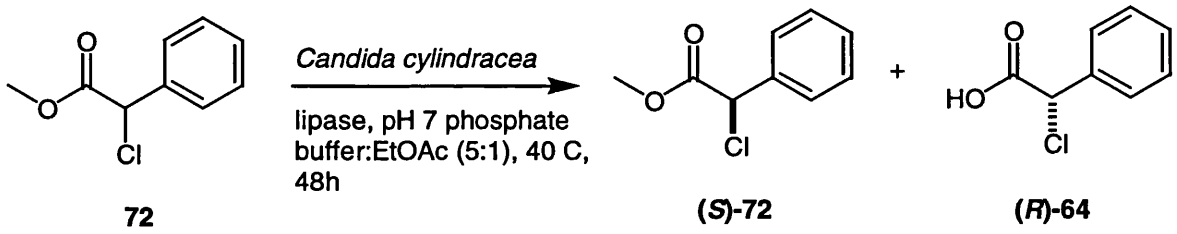
86

Table 26: The *dynamic kinetic* resolution of **72** using *Candida cylindracea* lipase and the immobilised Cl^- source **86**.

This was an excellent result but was marred by the reactions irreproducibility. We had found problems with the effectiveness of the resin in acid vs. ester studies and felt that these were playing a role in our DKR reaction. The best results were gained when freshly prepared resin was employed. It was also found that it was essential to wash the resin thoroughly with toluene, then ethanol, water and finally dichloromethane, which is effective at swelling the resin ready for use.

5.7 Pseudo-DKR

With both racemisation methodology of (*R*)-**72** and its enzymatic resolution having proved to be successful, and our DKR studies being somewhat irreproducible. We tried to couple these two methods together in series rather than in parallel. This as accomplished firstly by undertaking the *kinetic resolution* of **72** as shown in **Scheme 74**, and **table 25**.

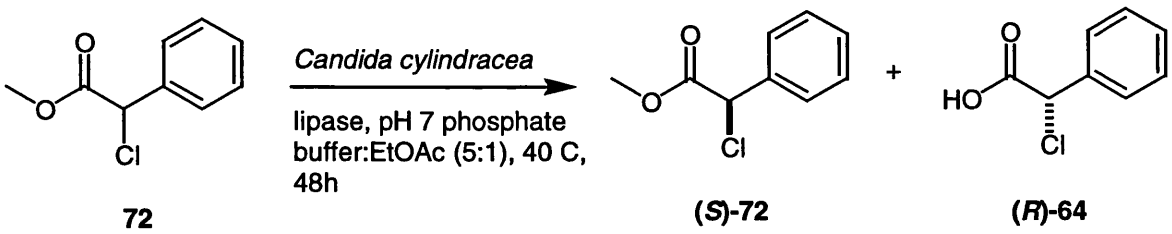


Scheme 74

Acid ee%	Ester ee%	Conversion (%by NMR)
90	75	42

Table 27: The *kinetic* resolution of **72** using *Candida cylindracea* lipase.

The enantiomerically enriched acid and esters were separated using classical extraction methods and the ester racemised using Merrifield resin bound phosphonium chloride **86** giving racemic ester **72** that was again subjected to enzyme hydrolysis giving acid (*R*)-**64** in 92%ee and 45% conversion. The overall process yielded (*R*)-**64** in 71% and 90% ee as shown in **Scheme 75**, and **Table 28**.



72 **(*S*)-72** **(*R*)-64**

Scheme 75

Acid ee%	Ester ee%	Conversion (% by NMR)	Total ee% acid	Yield
92	82	45	90	71

Table 28: Pseudo-*dynamic kinetic* resolution of **72** using *Candida cylindracea* lipase and the immobilised Cl^- source **86**.

The racemisation process could be repeated again recycling the enantiomerically enriched ester **(*S*)-72** to racemic material, which maybe subjected to another enzymatic resolution.

5.7 Conclusion

The racemisation of several α-chloro esters have been successful achieved under various conditions. We found that both increasing the temperature and addition of Lewis acid increased the rate of racemisation. Formation of the carboxylate was found to control the racemisation of the acid in comparison to its methyl ester **72** enabling studies towards the *dynamic kinetic resolution* of **64**.

Dynamic kinetic resolutions in phophate buffer were observed to inconclusive. Whereas reactions carried out in pH-controlled water showed limited *dynamic kinetic resolution* upon the use of phosphonium chlorides, the use of immobilised phosphonium chloride **86** overcame problems with enzyme/racemising agent incompatibility leading to a successful albeit irreproducible *dynamic kinetic resolution.*

A pseudo-DKR was successfully achieved on a 0.2 gram scale yielding 71% of (***R***)-**57** with 90% enantioselectivity after 4 days.

5.9 References

1 G. A. Slough, *Tetrahedron Lett.*, 1993, **34**, 6825.

2 K. Koh and T. Durst, *J. Org. Chem.*, 1994, **59**, 4683.

3 S. Caddick and K. Jenkins, *Chem. Soc. Rev.*, 1996, 447.

4 M. M. Jones, 'Enzymatic kinetic resolutions combined with racemisation techniques', PhD, Bath University, Bath, 1998.

5 M. M. Jones and J. M. J. Williams, *J. Chem. Soc., Chem Commun.*, 1998, 2519.

Section 6

Experimental

6.1 General Procedures

^{1}H NMR spectra were recorded in $CDCl_3$, unless otherwise stated, on a Jeol GX270 and Jeol GX400 spectrometers. Residual protic solvent $CHCl_3$ (δH = 7.26 ppm) was used as an internal reference. Coupling constants were measured in Hertz. ^{13}C NMR spectra were recorded in $CDCl_3$, unless otherwise stated, at 100 MHz using the resonance of $CDCl_3$ (δ = t, 77.0 ppm) as an internal reference. Infrared spectra were recorded on Perkin Elmer 1605-FT-IR,were samples were recorded as films. Mass spectra were recorded on a Fisons NG-Autospec mass spectrometer. Microanalysis was determined in themicroanalytical laboratory at the University of Bath. Melting points were measured on a Büchi 535 melting point apparatus and are uncorrected. Optical rotations were measured with an AA-10 auto-optical activity polarimeter. Titrations were carried out on a Metler DL21 titrator.

GC chromatographs were recorded using a Fisons, GC 800 series using 110 KPa of air and 55 KPa of hydrogen gas. HPLC analysis was carried out using Thermo separation products, spectra series UV100 and P200.

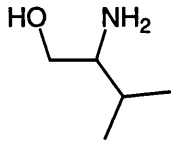
Flash Chromatography was carried out using Merck keiselgel 60 H under pressure unless otherwise stated. Analytical tlc was performed using Merck keiselgel $60F_{254}$ and visualised using UV light (254 nm), dips. Petrol refers to petroleum ether b.p. 40-60 °C and ether to diethyl ether.

Ether and tetrahydrofuran were distilled from sodium benzophenone ketyl; dichloromethane, acetonitrile and toluene from calcium hydride and triethylamine

from potassium hydroxide. Other solvents and reagents were purified using standard procedures[1].

6.2 Experimental for Section 2

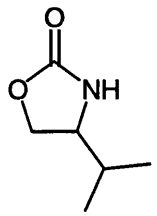
The preparation of 3-amino-3-methyl-butan-1-ol 33.[2, 3, 4]



33

Valine (1.0g, 8.5 mmol), was added slowly to a stirred solution of lithium aluminium hydride (0.7g, 17.9 mmol) in THF (30 mL) at 0 °C. After 1h, the solution was heated at reflux for 18h. Upon cooling to r.t. the reaction was poured into ice cold water (10 mL), sodium hydroxide solution (20 mL, 15% w/v), then water (10 mL). The resultant solution was diluted with toluene (50mL) and dried over 15h using a Dean-Stark trap, filtered and concentrated *in vacuo*. The resultant residue was distilled under pressure (bp 120 °C, 1 mm/Hg) giving **33** (0.5g, 57%) a white crystalline solid (mp 31-32 °C); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3389 (OH); (δ_H270MHz; $CDCl_3$); 3.61 (1H, dd, *J* 3.6, 10.4, C*H*OH), 3.32 (1H, dd, *J* 10.0, 10.68, C*H*OH), 2.75 (1H, s, CO*H*), 2.54-2.61 (1H, m, C*H*NH), 1.63 (1H, oct, *J* 7.1, C*H*CH_3) 0.95 (3H, d, *J* 6.72, CH_3), 0.92 (3H, d, *J* 7.0, CH_3); δ_C ($CDCl_3$); 65.5 (CH_2), 58.5 (CH), 31.0 (CH), 19.3 (CH_3), 18.5 (CH_3), m/z (CI^+) 104 (100%, MH^+).

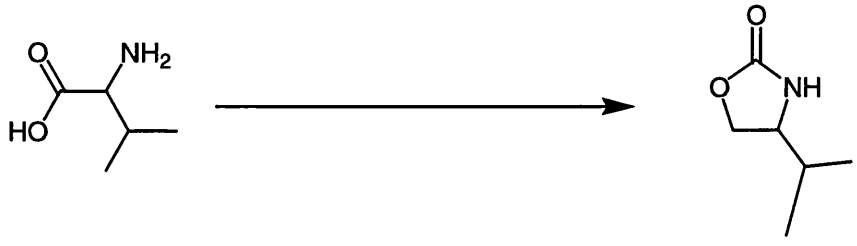
The preparation of 4-isopropyl-oxazolidinone 24. [5,6]



24

A solution of 2-amino-propan-3-ol (0.5 mL, 6.6 mmol) and diethyl carbonate (1.1 mL, 8.7 mmol) in toluene (10 mL) was dried using Dean-Stark apparatus over 1h. After cooling to r.t sodium methoxide (4mg, 0.07 mmol) was added and the reaction refluxed for 4h, filtered and concentrated *in vacuo*. The resultant residue was purified by chromatography (SiO_2, ethyl acetate:petrol, 5:1), giving **24** (0.3g, 76%) as a white crystalline compound (mp 75-76 °C); ν_{max}(film)/cm^{-1}; 3225 (NH), 1760 (CO); δ_H(400MHz; $CDCl_3$) 4.45 (1H, dd *J* 9.1, 8.3, C*H*O), 4.17 (1H, dd *J* 6.3, 8.5, C*H*O), 3.62 (1H, m, C*H*CH_2), 2.39 (1H, s, N*H*), 1.71-1.83 (1H, m C*H*CH_3), 0.95 (3H, d *J* 7.0, CHC*H*$_3$), 0.92 (3H, d *J* 6.9, CHC*H*$_3$); δ_C($CDCl_3$); 160 (C=O), 69.7 (CH_2), 58.4 (CH), 33.4 (CH), 18.2 (CH_3) 17.1 (CH_3); m/z (EI) 163 (80%, M^+).

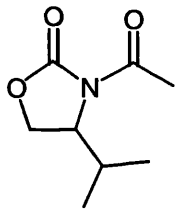
A novel 'one pot' preparation of Evans oxazolidinones.[7]



32 **24**

Valine (1.572g, 13.0 mmol) was added slowly to a stirred solution of lithium aluminium hydride (2.201g, 29.0 mmol) in THF (25 mL) at 0 °C for 1h. After 16h at reflux the reaction was poured into water (5 mL), sodium hydroxide (10 mL, 15% w/v), then water (5 mL) and filtered. Diethyl carbonate (3.16 mL, 27 mmol) and toluene (30 mL) were added and the resultant solution was dried using a Dean-Stark trap over 16h. The reaction was cooled to r.t and sodium methoxide added (4 mg, 0.07 mmol). After 4h at reflux the solution was filtered through celite and concentrated *in vacuo*, giving **29** (1.2012g, 54%) a colourless crystalline solid (mp 31-32 °C, chloroform:petrol). A proton NMR spectrum was identical to an authentic sample.

The preparation of 3-Acetyl-4-isopropyl-oxazolidinone 34. [8]

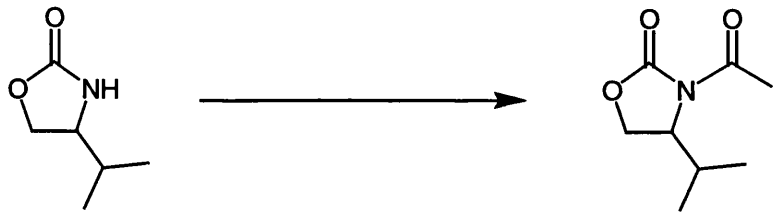


34

nBuLi (2.5M, 2.0 mL, 1.05 eq) was added to a solution of 4-isopropyl-oxazolidin-2-one in THF (10mL) at –78 °C. After 15 min, acetyl chloride (0.37mL, 5.2 mmol, 1.1eq) was added to the solution and the reaction was quenched with saturated ammonium chloride solution (10mL) and extracted into DCM (10mL). The organic layer was washed with 1M NaOH (5mL) and then brine (5mL), dried with $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography (ether:petrol, 95:5), affording a yellow oil (0.38g, 57%);

ν_{max}(film)/cm^{-1}; 1749, 1732 cm^{-1}; δ_H(200MHz; $CDCl_3$); 4.52-4.25 (m, OCH_2CHN), 2.45 (s, 3H, CH_3); 1.29 (d, *J* 6.8, 3H, CH_3), 1.25 (d, *J* 6.9, 3H, CH_3); δ_C($CDCl_3$); 170.1, 154.5 (C=O), 69.2 (CHO), 50.7 (CHN), 18.5 (CH_3), 14.4 (CH_3); m/z (EI) 144 (65%, M^+).

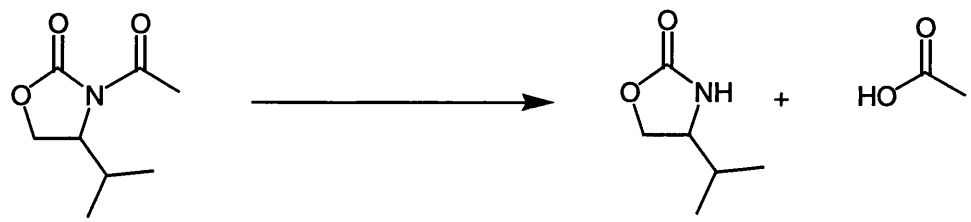
General Procedure for Enzyme Acylation of Oxazolidinones



24 **34**

Candida antartica Lipase (Boeringer-Mannheim,) (5 mg, 10% w/w) was added to a solution of oxazolidinone **24** (0.05g) in vinyl acetate (1 mL) at 40 °C. At a suitable time the resulting solution was filtered through celite and concentrated *in* vacuo, giving oxazolidinone and acetate respectively as observed by proton NMR.

General Procedure for Hydrolysis of Evans Auxiliaries

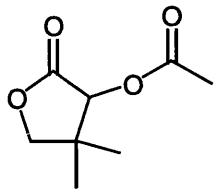


34 **24**

Candida antartica lipase (5 mg, 10% w/w) was added to a solution of acyl oxazolidinone **34** (0.05g) in phosphate buffer:*t*BuOMe 80:20 (1 mL) at 40°C. At a suitable time the resulting solution was filtered through celite, acidified with 0.1M HCl, extracted with ethyl acetate (3 × 5 mL). The extracts dried with $MgSO_4$ and concentrated *in* vacuo, giving oxazolidinone and acetate respectively as observed by proton NMR.

6.3 Experimental for Section 3.

The preparation of dihydro-4,4-dimethyl-(3H)-furan-2-one-3-yl-ethanoate34.[9]

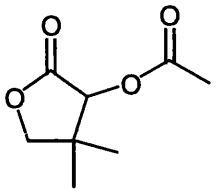


34

Acetic anhydride (1.2 mL, 13.05 mmol) was added to a solution of Pantolactone (1g, 7.68 mmol) and triethylamine (1.82 mL, 13.06 mmol) in dichloromethane (15 mL) at r.t. After 17h the reaction was poured into HCl (10 mL, 2M) and extracted with EtOAc (3 × 10 mL). The extract was dried with $MgSO_4$, and concentrated *in vacuo*. The resultant residue was purified by column chromatography (SiO_2, 40:60 ethyl acetate:petrol) giving **34** (1.24g, 95%) a yellow oil;

ν_{max}(film)/cm^{-1} 1798 (C=O), 1760 (C=O), 1408 (CO), 1094 (CO); δ_H(400MHz, $CDCl_3$); 5.33 (1H, s, C*H*O), 4.01 (2H, s, OCH_2), 2.17 (3H, s, COCH_3), 1.17 (3H, s, CH_3), 1.07 (3H, s, CH_3); δ_C($CDCl_3$) 172.5 (C=O), 170.2 (C=O), 75.4 (CH), 40.5 (CH_2), 23.7 (CH_3), 20.5 (CH_3); m/z (CI+) 173 (88%, MH^+).

The preparation of name dihydro-4,4-dimethyl-(3H)-furan-2-one-3-yl-ethanoate (*R*)-34

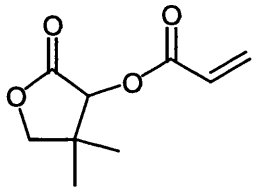


(*R*)-34

The reaction of (R)-Pantolactone under the same conditions gave **(*R*)-34** (1.1g, 90%) as a colourless oil. The proton NMR was identical to an authentic sample.

$[\alpha]_D$ –13.3 (c. 0.3 in chloroform).

Preparation of dihydro-4,4-dimethyl-(3H)-furan-2-one-3-yl-propenoate 40.[9,10,11,12]



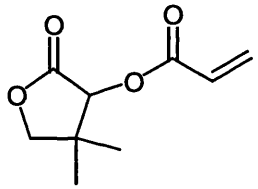
40

Acryloyl chloride (5.0 mL, 62.5 mmol), was added to a solution of pantolactone (6.5g, 50.0 mmol) and triethylamine (10.5 mL, 75.0 mmol) in dichloromethane (75 mL) at -24 °C. After 5h at -24 °C the reaction was poured into 1.0 M HCl (40mL) and extracted with ethyl acetate (3 × 10 mL). The extracts were washed with $NaHCO_3$ (40

mL) brine (40 mL) and dried with $MgSO_4$ and concentrated *in vacuo*. The resultant residue was distilled (bp 90 °C, 1mm/Hg) giving **34** (6.79g, 74%) a colourless oil;

ν_{max}(film)/cm^{-1} 2970, 2935, 2911, 2879 (C=C), 1792 (lactone), 1737 (lactone), 1634 (C=O); δ_H(400MHz; $CDCl_3$); 6.53 (1H, dd, *J* 1.2 and 17.4 Hz, =C*H*), 6.23 (1H, dd, *J* 11.2 and 17.3 Hz, =C*H*), 5.98 (1H, dd, *J* 1.3 and 11.5, COC*H*=), 5.45 (1H, s, CH), 4.09 (1H, d, *J* 9.0 Hz, CH_2), 4.06 (1H, d, *J* 9.0 Hz, CH_2), 1.14, 1.23 (3H, s, CH_3); δ_C($CDCl_3$); 172.5 (C=O), 165.2 (CO), 133.3 (CH_2), 127.1 (CH), 77.5 (CH_2), 75.2 (CH), 41.4 (C), 20.2, 23.2 (CH_3); m/z (EI) 55 (100%, M^+). $C_9H_{12}O_4$ requires: C, 58.69%; H, 6.57%. Found: C, 58.70%; H, 6.63%.

Preparation of dihydro-4,4-dimethyl-(3H)-furan-2-one-3-yl-propenoate (*R*)-40.[9,12]

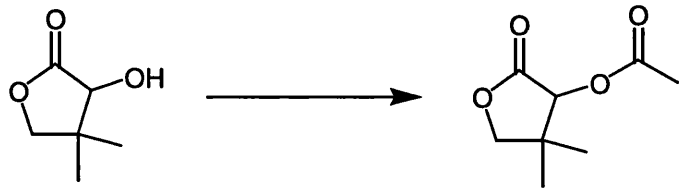


(*R*)-40

The reaction of (R)-pantolactone (6.5g, 50 mol) under the same conditions gave (6.79g, 74%) as a colourless oil. The proton NMR was identical to that of an authentic sample.

$[\alpha]_D$ -10 (c. 2 in chloroform).

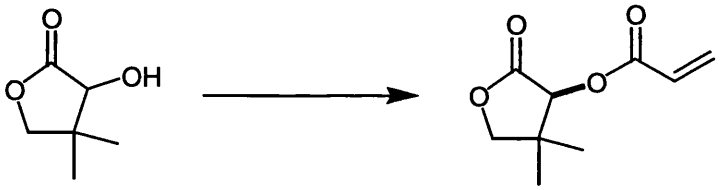
Enzymatic acylation of Pantolactone



35 **49**

Pseudomonas cepacia Lipase (Altus 20, CLEC) (20 mg, 20% w/w) was added to a solution of dried Pantolactone (0.1g, 0.77 mmol) in vinyl acetate (1 mL) at r.t. At a suitable time as judged by TLC analysis the resulting solution was filtered through celite and concentrated *in* vacuo, giving alcohol and acetate respectfully as observed by proton NMR.

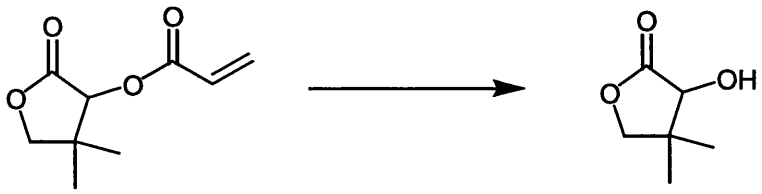
Enzyme catalysed acrylation of Pantolactone



35 **40**

Pseudomonas cepacia Lipase (Altus 20, CLEC) (5 mg, 10% w/w) was added to a solution of dried Pantolactone (0.05g, 0.38 mmol) and vinyl acrylate (79µl, 1.152 mmol) in *t*BuOMe (1 mL) at 25 °C. At a suitable time as judged by TLC analysis the resulting solution was filtered through celite and concentrated *in* vacuo, giving alcohol and acrylate respectively as observed by proton NMR.

The enzymatic hydrolysis of pantolactone acrylate



40 **35**

Pseudomonas cepacia Lipase (Altus 20, CLEC) (2 mg, 20% w/w) was added to a solution of pantolactone acrylate (0.01g, mmol) in phosphate buffer:*t*BuOMe 80:20 (1 mL) at r.t. At a suitable time as judged by TLC analysis the resulting solution was filtered through celite, acidified with 01M HCl, extracted with ethyl acetate (3 × 5 mL). The extracts dried with $MgSO_4$ and concentrated *in* vacuo, giving alcohol and acetate respectively as observed by proton NMR.

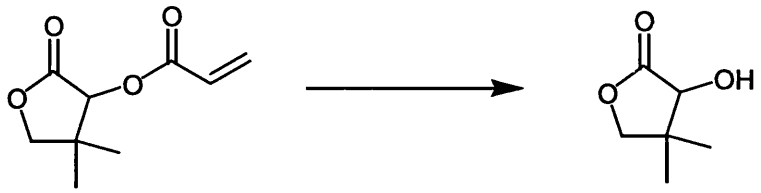
The enzymantic transesterification of pantolactone acrylate, method A

40 **35**

Pseudomonas cepacia Lipase (Altus 20, CLEC) (2 mg, 20% w/w) was added to a solution of pantolactone acrylate (0.01g, mmol) in methanol (1 mL) at r.t. At a suitable time as judged by TLC analysis the resulting solution was filtered through celite, acidified with 01M HCl, extracted with ethyl acetate (3 × 5 mL). The extracts

dried with $MgSO_4$ and concentrated *in* vacuo, giving alcohol and acetate respectively as observed by proton NMR.

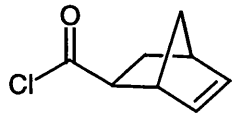
The enzymantic transesterification of pantolactone acrylate, method B



40 **35**

Pseudomonas cepacia Lipase (Altus 20, CLEC) (2 mg, 20% w/w) was added to a solution of pantolactone acrylate (0.01g, mmol), methanol (10 eq, mL) in *t*BuOMe (1 mL) at r.t. At a suitable time as judged by TLC the resulting solution was filtered through celite, acidified with 01M HCl, extracted with ethyl acetate (3 × 5 mL). The extracts dried with $MgSO_4$ and concentrated *in* vacuo, giving alcohol and acetate respectively as observed by NMR analysis.

The preparation of bicyclo [2.2.1]hept-5-ene-2-carbonyl chloride 88

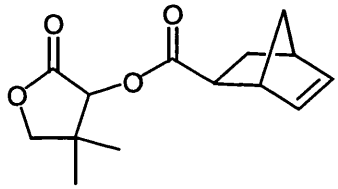


88

Norbornene carboxylic acid (0.48g, 3 mmol) was dissolved in thionyl chloride (10 mL) and heated to reflux for 16h. The resultant solution was distilled under pressure (92-94 °C, 1mm/Hg) giving **88** (0.4997g 85%) a yellow oil;

ν_{max}(film)/cm^{-1} 1732 (C=O).

The preparation of bicyclo[2.2.1]hept-5-ene-2-carboxylic acis 4,4-dimethyl-2-oxo-tetrahydro-furan-3-yl ester 41. [9,11]



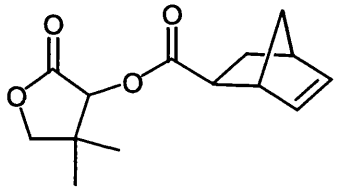
41

Acid chloride (0.132g, 0.847 mmol) **88**, was added to solution of pantolactone (0.1g, 0.77 mmol), triethyl amine (0.119 mL, 0.847 mmol) in dichloromethane (5 mL) at 0 °C. After 16h at r.t. the reaction was poured into 0.1M HCl (10mL) and extracted with ethyl acetate (3 × 10 mL). The extracts were, washed with brine and dried with $MgSO_4$ and concentrated *in vacuo*. The resultant residue was recrystallised giving **41**

(0.12g, 73%) a colourless crystalline solid (mp 95-97 °C, ethyl acetate:hexane) as a mixture of diastereoisomers. The de was analysed by chiral gas chromatography (see appendix II);

ν_{max}(film)/cm^{-1} 2968/2934/2876 (C=C), 1792 (C=O), 1734 (C=O), 1072/1012 (C-O); δ_H(400MHz; $CDCl_3$); 6.24 (1H, dd, *J* 5.9, 3.1, =C*H*), 5.89 (1H, dd, *J* 5.9, 3.1, =C*H*), 5.31 (1H, s, C*H*O), 4.04 (1H, d, *J* 9.0, OC*H*H), 4.00 (1H, d, *J* 9.0, OC*H*H), 3.25 (1H, m, C*H*) 3.14 (1H, d of app. t, *J* 7.0, 3.9, C*H*CO_2), 2.93 (1H, m, C*H*), 1.94 (1H, ddd, *J* 3.9, 9.4, 12.1), 1.44-1.50 (m, 2H, C*H*), 1.31 (1H, d, CH_2), 1.13 (3H, s, CH_3), 1.16 (3H, s, CH_3); δ_C($CDCl_3$); 174.2 (C=O), 173.5 (C=O), 139.4 (=CH), 132.5 (=CH), 76.6 (CH_2), 75.2 (CHO), 50.5 (CH_2), 46.4 (CH), 43.7 (CH), 43.5 (C(CH_3)$_3$), 29.8 (CH_2), 24.7 (CH_3), 21.8 (CH_3); m/z (EI) 252 (47%, M^+).

The preparation of (*R*)-Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid 4,4-dimethyl-2-oxo-tetrahydro-furan-3-yl ester (*R*)-41. [9]

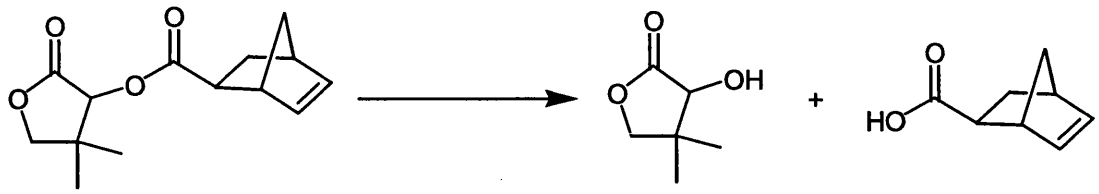


(*R*)-41

The reaction of (*R*)-Pantolactone under the same conditions gave **(*R*)-41** (0.13g, 79%) as a colourless crystalline solid. The proton NMR was identical to an authentic sample.

$[\alpha]_D$ –8.0 (c 2 in methanol).

The enzymatic hydrolysis of bicyclo[2.2.1]hept-5-ene-2-carboxylic acids 4,4-dimethyl-2-oxo-tetrahydro-furan-3-yl ester 41.

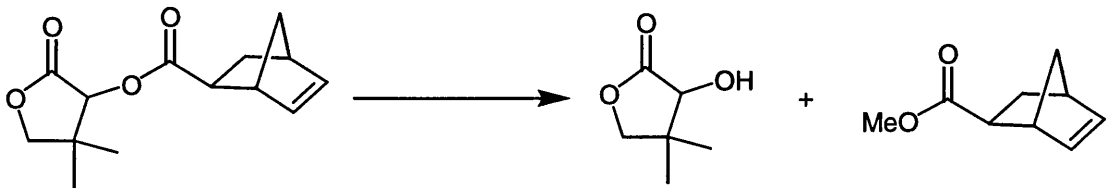


41 **35**

Pseudomonas cepacia Lipase (Altus 20, CLEC) (2 mg, 20% w/w) was added to a solution of pantolactone acrylate (0.01g, mmol) in phosphate buffer:*t*BuOMe 80:20 (1 mL) at r.t. At a suitable time as judged by TLC the resulting solution was filtered through celite, acidified with 01M HCl, extracted with ethyl acetate (3 × 5 mL). The extracts dried with $MgSO_4$ and concentrated *in* vacuo, giving alcohol and acetate respectively as observed proton NMR analysis.

1 eq. of analine was used in some experiments (see section 3).

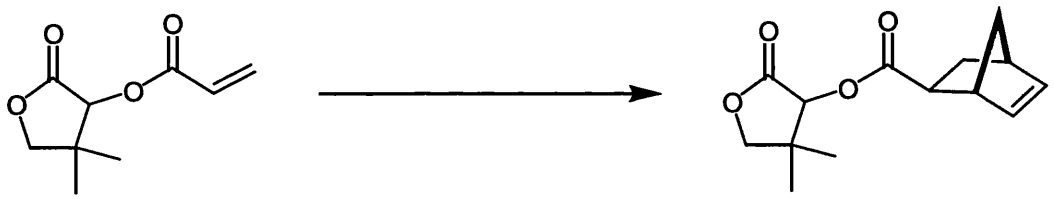
The enzymatic Transesterification of bicyclo[2.2.1]hept-5-ene-2-carboxylic acid 4,4-dimethyl-2-oxo-tetrahydro-furan-3-yl ester 41.



41 **35**

Pseudomonas cepacia Lipase (Altus 20, CLEC) (2 mg, 20% w/w) was added to a solution of pantolactone acrylate (0.01g, mmol) in methanol (1 mL) at r.t. At a suitable time the resulting solution was filtered through celite, acidified with 01M HCl, extracted with ethyl acetate (3 × 5 mL). The extracts dried with $MgSO_4$ and concentrated *in* vacuo, giving alcohol and acetate respectively as observed by proton NMR.

Asymmetric Diels Alder Reactions using Pantolactone Chiral Auxiliary. [13]

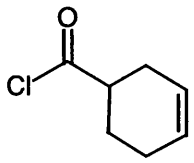


40 **41**

Titanium chloride (0.03 mL, 0.2715 mmol) was added to a solution of pantolactone acrylate (0.5g, 0.2715 mmol) in dry dichloromethane:petrol ether (7:1, 4.4:0.6 mL) at –10 °C. After 30 min, freshly cracked cyclopentadiene (0.2262g, 3.53 mmol) was added and the solution stirred for 1h, upon which pellets of $Na_2CO_2.10\ H_2O$ (0.3g) were added, the solution filtered through celite and concentrated *in vacuo*. The resultant residue was re-crystallised giving **41** (0.647g, 95%) colourless crystals (mp 96-98 °C, ethyl acetate:petrol), the diastereomeric excess was determined by chiral gas chromatography (see appendix II).

de% 83 (Gas chromatography see appendix II).

The preparation of cyclohex-3-ene carbonyl chloride 89

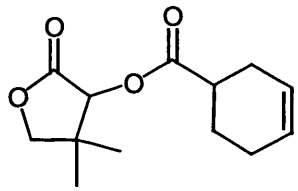


89

cyclohexene carboxylic acid (0.48g, 3.8 mmol) was dissolved in thionyl chloride (10 mL) and refluxed for 16h. The resultant solution was distilled under pressure (133-134, 1mm/Hg Torr) giving **89** (0.46g 85%) a yellow oil;

ν_{max}(film)/cm^{-1} 1704 (C=O).

The preparation of name cyclohex-3-ene carboxylic acid 4,4-dimethyl-oxo-tetrahydrofuran-3-yl ester 55.[13]



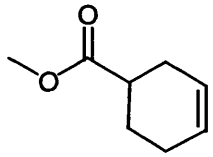
55

Acid chloride (1.2g, 8.5 mmol) was added to a solution of Pantolactone (1.0g, 7.6 mmol), triethylamine (0.19 mL, 8.5 mmol)) in dichloromethane (25 mL) at 0 °C. After 5h at r.t, the reaction was poured into HCl (50 mL, 2M) and extracted with ethyl acetate (3 × 30 mL). The extracts were washed with saturated sodium bicarbonate (30 mL), brine (30 mL), dried with $MgSO_4$ and concentrated *in vacuo*. The resultant

residue was kugelrohr distilled (80°C, 1mm/Hg) giving **55** (82%, 6.2 mmol) as a colorless oil;

ν_{max}(film)/cm^{-1} 2935 (C=C), 1791 (C=O), 1746 (C=C), 1152 (C-O); δ_H(200MHz; $CDCl_3$); 5.73 (1H, d, *J* 5.9, =C*H*), 5.45, (1H, s, =C*H*), 4.01 (2H, d, *J* 4.2, CH_2), 2.95-3.12 (1H, m, CH_2), 2.11-2.28 (6H, m, CH_2), 1.71-1.89 (2H, m, CH_2), 1.2 (3H, s, CH_3), 1.1 (3H, s, CH_3); δ_C($CDCl_3$); 174.2 (C=O), 172.8 (C=O), 137.7 (=CH), 132.4 (=CH), 76.5 (CH_2), 74.4 (CH), 40.5 (C), 27.7 (CH_2), 25.2 (CH_2), 24.5 (CH_2), 23.1 (CH_2), 21.1 (CH_3), 20 (CH_3); m/z (EI) 238 (100%, M^+); (found: M^+NH_4, 256.1549. $C_{13}H_{18}O_4$ requires *M*, 238.2732).

The preparation of name cyclohex-3-ene carboxylic acid methyl ester 90.[14]

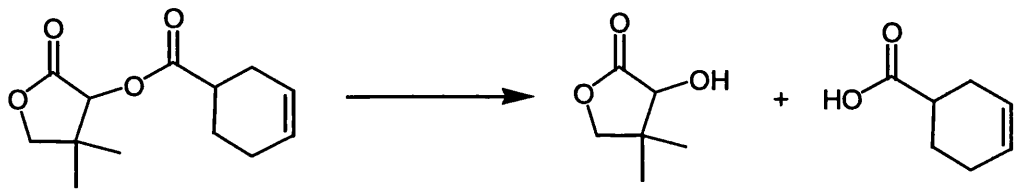


90

Three drops of concentrated hydrolchloric acid were added to a stirred solution of cyclohexene carboxyllic acid (0.2g, 1.6 mmol) in methanol (5 mL) at r.t. After 16h the reaction was poured into a saturated sodium bicarbonate solution (10 mL) and extracted with ethyl acetate (3 × 10 mL). The extract was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was kugelrohr distilled (82-83 °C 1mm/Hg) to give **90** (0.20g, 90%) a colourless oil;

ν_{max}(film)/cm^{-1} 3024/2950/2842 (C=C), 1733 (C=O), 1436 (C-O), 1167 (C-O); δ_H(400MHz; $CDCl_3$); 5.71-5.85 (2H, m, =C*H*), 3.62 (s, 3H, CH_3), 2.51-2.65 (1H, m, C*H*O), 2.31-2.25 (2H, m, CH_2), 2.01-2.17 (2H, m, CH_2), 2.05 (2H, m, CH_2), 1.61-1.75 (1H, m, CH_2); δ_C($CDCl_3$); 176.3 (C=O), 126.8 (=CH), 125.4 (=CH), 51.9 (CH_3), 39.6 (C=OCH), 27.9 (CH_2), 25.5 (CH_2), 24.8; m/z (CI) 141 (60%, M^+).

The enzymatic hydrolysis of cyclohex-3-ene carboxylic acid 4,4-dimethyl-oxo-tetrahydrofuran-3-yl ester 55.

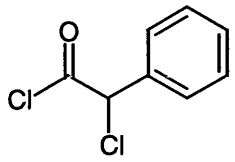


55 **35**

Pseudomonas cepacia Lipase (Altus 20, CLEC) (3 mg, 10% w/w) was added to a solution of (25mg, mmol) in phosphate buffer:*t*BuOMe (1 mL, 80:20) at r.t. At a suitable time the resulting solution was filtered through celite, acidified with 01M HCl, extracted with ethyl acetate (3 × 5 mL). The extracts dried with $MgSO_4$ and concentrated *in* vacuo, giving alcohol and acid respectfully.

6.4 Experimental for Sections 4 and 5

The preparation of α-chloro phenyl acetyl chloride 71.[15]

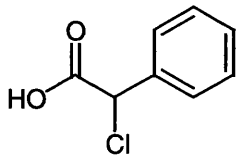


71

Mandelic acid (32.00g, 0.21 mol) was dissolved in thionyl chloride (250 mL) and heated to reflux for 16h. The resultant solution was distilled under pressure giving **71** (37.59g. 95%) a yellow oil, (bpt 66 °C, 2mm/Hg);

ν_{max}(film)/cm^{-1} 1804.3 (C=O), 711.9 (CCl).

The preparation of α-chloro phenyl acetic acid 57.[16]



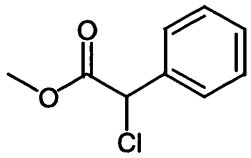
64

Water (20 mL) was slowly added to a solution of α-chloro-phenyl-acetyl-chloride **71** in ethyl acetate (50 mL) and was stirred at r.t. for 1h. The aqueous layer was extracted with ethyl acetate (3 × 25 mL), dried with $MgSO_4$ and concentrated *in vacuo*. The resultant residue was purified by column chromatography (SiO_2, ethyl

acetate-hexane, 20:80) to give **64** (15.8g, 52%) a white solid, mp 75-77°C (from ethyl acetate-hexane);

ν_{max}(film)/cm^{-1} 3064 (COOH), 1702 (C=O), 696 (CCl); δ_H(400MHz; $CDCl_3$); 10.9 (1H, s, COOH), 7.51-7.65 (2H, m, Ph), 7.40-7.55 (3H, m, Ph), 5.38 (1H, s, C*H*Ph); δ_C($CDCl_3$); 174.5 (C=O), 135.1, (CH), 129.8 (CH), 129.2 (CH), 128.2 (CH), 59.0 (CHCl); *m/z* (FAB) 171 (97%, M^+), 125 (87%).

The preparation chlorophenyl acetic acid methyl ester 72.[17,18]

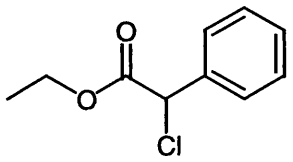


72

α-chloro-phenyl-acetyl-chloride (5.0g, 0.027 mol) was added slowly to a solution of triethyl amine (4.45 mL, 0.032 mol) in methanol (30 mL) at 0 °C. After 3h at r.t. the reaction was poured into water (50 mL) and extracted with ethyl acetate (3 × 50 mL) the extract dried with $MgSO_4$ and concentrated *in vacuo*. The resultant residue was distilled under pressure giving **72** (4.7g, 95%) a colourless oil (bp 70 °C at 3mm/Hg);

ν_{max}(film)/cm^{-1} 1756 (C=O), 1455 (CO), 1163 (CO); δ_H(400MHz; $CDCl_3$); 7.51-7.62 (2H, m, Ph), 7.31-7.5 (3H, m, Ph) 5.32 (1H, s, C*H*Cl), 3.82 (1H, s, CH_3); δ_C($CDCl_3$); 168.9 (C=O), 135.9 (CH), 129.5 (CH), 129.1 (CH), 128.1 (CH), 59.3 (CHCl), 53.7 CH_3); *m/z* (EI) 184 (23%, M^+), 125 (97%).

The preparation chlorophenyl acetic acid ethyl ester 73. [64]

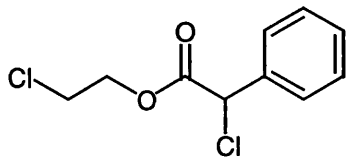


73

α-chloro-phenyl-acetyl-chloride (5.0g, 0.027 mol) was added slowly to a solution of triethyl amine (4.45 mL, 0.032 mol) in ethanol (30 mL) at 0 °C. After 4h at r.t. the reaction was poured into water (50 mL) and extracted with ethyl acetate (3 × 50 mL) the extract dried with $MgSO_4$ and concentrated *in vacuo*. The resultant residue was distilled under pressure giving **73** (4.9g, 95%) a colourless oil (bp 98 °C at 1.5 mm/Hg);

ν_{max}(film)/cm^{-1} 1752 (C=O), 1455 (C-O), 1158 (C-O), 727 (CCl); δ_H(400MHz; $CDCl_3$); 7.51-7.65 (2H, m, Ph), 7.42-7.35 (3H, m, Ph) 5.38 (1H, s, C*H*Cl), 4.26-4.35 (2H, m CH_2); 1.25 (3H, t, *J* 7.1, CH_3); δ_C($CDCl_3$); 168.4 (C=O), 136.1 (CH), 129.4 (CH), 129.0 (CH), 128.1 (CH), 62.8 (CHCl), 59.5 (CH_2), 14.4 (CH_3); *m/z* (CI) 199 (89%, MH^+), 178 (66%), 163 (100%), 102 (73%); (found: MH^+, 199.05260. $C_{10}H_{11}ClO_2$ requires *M*H, 199.0527).

The preparation chlorophenyl acetic acid 2-chloroethyl ester 74

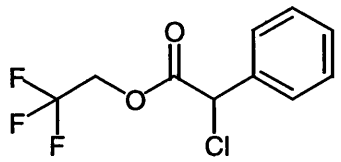


74

α-chloro-phenyl-acetyl-chloride (5.0g, 0.027 mol) was added slowly to a solution of triethyl amine (4.45 mL, 0.032 mol), 2-chloroethanol (2.58g, 0.032 mol) in THF (30 mL) at 0 °C. After 5h at r.t. the reaction was poured into water (50 mL) and extracted with ethyl acetate (3 × 50 mL) the extract dried with $MgSO_4$ and concentrated *in vacuo*. The resultant residue was distilled under pressure giving **74** (5.3g, 91%) a pale yellow oil (bp 120 °C at 1.5 mm/Hg);

ν_{max}(film)/cm^{-1} 1756 (C=O), 1455 (CO), 1161 (CO), 728 (CCl); δ_H(400MHz; $CDCl_3$); 7.51-7.45 (2H, m, Ph), 7.41-7.35 (3H, m, Ph) 5.38 (1H, s, C*H*Cl), 4.41-4.35 (2H, m, CH_2O); 3.7 (2H, t, *J* 6.5, C*H*Cl) δ_C($CDCl_3$); 168.1 (C=O), 135.5 (CH), 129.6 (CH), 129.1 (CH), 128.1 (CH), 65.9 (CHCl), 59.1 (CH_2) 41.4 (CH_2Cl); ***m/z*** (EI) 233 (100%, M^+); (found: M^+NH_4, 250.0402. $C_{10}H_{10}Cl_2O_2$ requires *M*, 233.0891). (Found: C, 51.2; H, 4.3. $C_{10}H_{10}O_2Cl_2$ requires C, 51.5 ; H, 4.3 %).

The preparation chlorophenyl acetic acid trifluoroethyl ester 75.

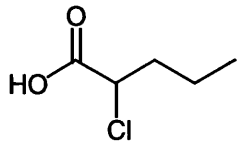


75

α-chloro-phenyl-acetyl-chloride (5.0g, 0.027 mol) was added slowly to a solution of triethyl amine (4.5 mL, 0.032 mol), trifluoroethanol (3.2g, 0.032 mol) in THF (30 mL) at 0 °C. After 5h at r.t. the reaction was poured into water (50 mL) and extracted with ethyl acetate (3 × 50 mL) the extract dried with $MgSO_4$ and concentrated *in vacuo*. The resultant residue was distilled under pressure giving **75** (4.5g, 70%) a colourless oil (bp 92 °C at 1.5mm/Hg);

ν_{max}(film)/cm^{-1} 1772 (C=O), 1455 (CO), 1171 (CO); 7.29 (CCl), 695 (CF_3); δ_H(400MHz; $CDCl_3$); 7.51-7.45 (2H, m, Ph), 7.41-7.35 (3H, m, Ph) 5.46 (1H, s, C*H*Cl), 4.41-4.65 (2H, 3F, m, CH_2 CF_3); δ_C($CDCl_3$); 167.1 (C=O), 134.9 (CH), 129.8 (CH), 129.2 (CH), 128.1 (CH), 62.0 (CHCl), 58.6 (CH_2), 14.5 (CF_3); ***m/z*** (EI$^+$) 252 (100%, M$^+$); (found: M$^+$, 252.0167. $C_{10}H_8O_2ClF_3$ requires *M*, 252.5832). (Found: C, 47.2; H, 3.23. $C_{10}H_8O_2ClF_3$ requires C, 47.5; H, 3.19 %).

The preparation of 2-chloro pentanoic acid 78.[19]

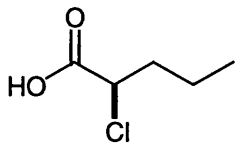


78

Sodium nitrite (2.97g, 0.043 mol) was added in portions (slowly) to a solution of nor-valine in etherial hydrochloric acid at 0 °C. After 16h at r.t. the reaction was poured into water and extracted with dichloromethane (3 × 10 mL). The extract was washed with sodium bicarbonate and with brine, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was Kugelrohr distilled (132 °C, 2 Hg/mm) giving **78** (3.67g, 71%) as a colourless oil.

ν_{max}(film)/cm^{-1} 3170 (COOH), 1713 (C=O), 772 (C-Cl), 751 (C-Cl); δ_H(400MHz; $CDCl_3$); 9.1 (1H, s, COO*H*), 2.01-1.97 (1H, m, CH_2), 1.56-1.45 (2H, m, CH_2), 1.05-1.12 (3H, t, *J* 7.4, CH_3.); δ_C($CDCl_3$); 175.2 (C=O), 57.0 (CHCl), 36.7 (CH_2), 19.3 (CH_2), 13.3 (CH_3) m/z (CI) 136.9 (72%, M^+).

The preparation of (*R*)-2-chloro pentanoic acid (*R*)-78

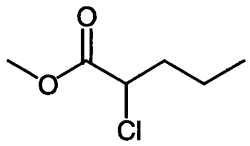


(*R*)-78

The reaction of (R)-norvaline (1.0g, 8.5 mmol) under the same conditions gave (1.1g, 94%) as a colourless oil.

$[\alpha]_D$ 13.7 (c. 0.1 in methanol).

The preparation of 2-chloro pentanoic acid methyl ester 79.[20,21]

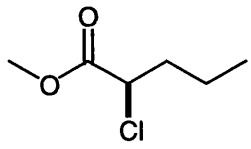


79

Three drops of concentrated hydrolchloric acid were added to a stirred solution of 2-chloro pentanoic acid (1.0g, 8.33 mmol) in methanol (10 mL) at r.t. After 16h the reaction was poured into a saturated sodium bicarbonate solution (10 mL) and extracted with ethyl acetate (3 × 10 mL). The extract was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was kugelrohr distilled giving **79** (1.1g, 82%) a colourless oil.

ν_{max}(film)/cm^{-1}; 2963 (C-H), 1652 (C=O), 1464 (C-O), 1171 (C-O), 769 (C-Cl); δ_H(400MHz; $CDCl_3$); 4.1 (1H, t, *J* 7.2 CHCl), 3.7 (3H, s, CH_3), 2.25-1.65 (2H, m, CH_2CHCl), 1.66-1.12 (2H, m, CH_2), 0.95 (3H, t, *J* 7.1, CH_3); δ_C($CDCl_3$); 159.1 (C=O), 56.6 (CHCl), 44.4 (CH_3), 38.4 (CH_3), 38.4 (CH_2), 35.0 (CH_2), 27.0 (CH_2), 19.8 (CH_3); m/z (CI) 166.1 (95%, M^+).

The preparation of (*R*)-2-chloro pentanoic acid (*R*)-79

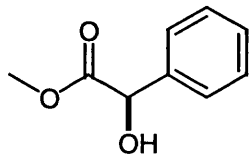


79

The reaction of (R)-norvaline (0.1g, 0.73 mmol) under the same conditions gave (0.1g, 91%) as a colourless oil. The proton NMR was identical to an authentic sample.

$[\alpha]_D$ –24.3 (c. 0.1 in methanol).

The prepartion of (*R*)-α-hydroxyphenylacetic acid methyl ester (*R*)-82.[22,23]

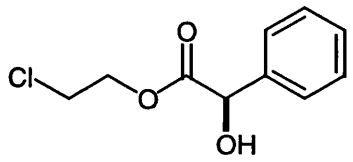


(*R*)-82

Three drops of concentrated hydrolchloric acid were added to a stirred solution of mandelic acid (5.0g, 32.9 mmol) in methanol (50 mL) at r.t. After 16h the reaction was poured into a saturated sodium bicarbonate solution (25 mL) and extracted with ethyl acetate (3 × 25 mL). The extract was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was kugelrohr distilled (140 °C, 1mm/Hg) to give **(*R*)-82** (5.1g, 93%) a colourless oil.

ν_{max}(film)/cm^{-1}; 3447 (OH), 3033/2952 (CH), 1741 (C=O), 1454/1437/1094/1067 (C-O), 733/699 (C-Cl); δ_H(400MHz; $CDCl_3$); 7.41-7.32 (2H, m, Ph), 7.31-7.4 (3H, m, Ph), 5.26 (1H, s, C*H*Cl), 4.25 (1H, s, O*H*), 3.77 (3H, s, CH_3); δ_C($CDCl_3$); 173.5 (C=O), 138.0 (C), 128.2 (CH), 128.0 (CH), 126.3 (CH), 72.7 (CHCl), 52.6 (CH_3), m/z (FAB+) 167.0 (51%, MH^+); $[\alpha]_D$ –80.1 (c. 3.1 in chloroform).

The prepartion of (*R*)-α-hydroxyphenylacetic acid 2-chloroethyl ester (*R*)-83



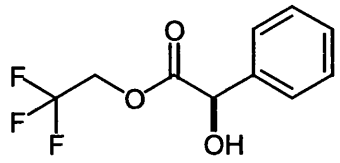
(*R*)-83

Three drops of concentrated hydrolchloric acid were added to a stirred solution of mandelic acid (1.0g, 6.57 mmol) in 2-chloro ethanol (10 mL) at r.t. After 16h the reaction was poured into a saturated sodium bicarbonate solution (10 mL) and extracted with ethyl acetate (3 × 10 mL). The extract was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was kugelrohr distilled (95 °C, 1mm/Hg) to give **(*R*)-82** (0.68g, 44%) a colourless oil.

ν_{max}(film)/cm^{-1}; 3424 (OH), 3061/2986/2903 (=CH), 1730 (C=O), 1453 (C-O), 1183 (C-O), 730 (C-Cl), 698 (C-Cl); δ_H(400MHz; $CDCl_3$); 7.45-7.32 (1H, d, *J* 7.8, Ph), 7.31-7.25 (4H, Ph), 5.28 (1H, s, CO*H*), 4.22 (2H, t, *J* 8.1, CH_2), 4.15 (2H, t, *J* 8.2, CH_2Cl); δ_C($CDCl_3$); 173.4 (C=O), 138.1/128.3/128.2/126.3 (CH(Ph)), 72.8 (C*H*(OH)), 62.1 (CH_2), 14..0 (CH_2Cl); m/z (EI) 214.1 (100%, M^+); (found: 232.0742, M^+NH_4,. $C_{10}H_{11}ClO_3$ requires M^+NH_4, 232.0740).

$[\alpha]_D$ −106.2 (c. 3.8 in chloroform).

The prepartion of (*R*)-α-hydroxyphenylacetic acid 2-trifluoroethyl ester (*R*)-84



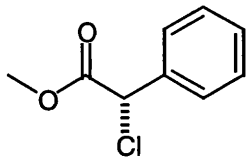
(*R*)-84

Three drops of concentrated hydrolchloric acid were added to a stirred solution of mandelic acid (1.0g, 6.57 mmol) in trifluoro ethanol (10 mL) at r.t. After 16h the reaction was poured into a saturated sodium bicarbonate solution (10 mL) and extracted with ethyl acetate (3 × 10 mL). The extract was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was kugelrohr distilled (90 °C, 1mm/Hg) to give **(*R*)-82** (0.68g, 44%) a colourless oil.

ν_{max}(film)/cm^{-1}; 4416 (OH), 3071/3033/2974 (=CH), 2357/2337 (C-H), 1754 (C=O), 1454/1428/1102/1070 (C-O); δ_H(400MHz; $CDCl_3$); 7.45-7.32 (4h, m, Ph), 5.30 (s, 1H, C*H*OH), 4.63-4.57 (2H, m, CH_2), 4.42-4.35 (3H, m, CF_3), 3.47 (1H, s, O*H*); δ_C($CDCl_3$); 171.9 (C=O), 137.0/128.8/128.7/126.37 (CH (Ph)), 72.8 (C-OH), 61.4 (CH_2), 61.0 (CF_3); m/z (EI) 234 (100%, M^+); (found: M^+NH_4, 252.0850 $C_{10}H_9O_3F_3$ requires M^+NH_4, 252.0848).

$[\alpha]_D$ −112.7 (c. 3.4 in chloroform).

The preparation (*S*)-α-chlorophenyl acetic acid methyl ester (*S*)-74

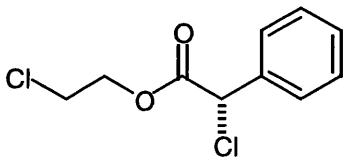


(*S*)-74

Thionyl chloride (0.759 mL, 18 mmol, 1eq) was added to **(*R*)-82** (3g, 18 mmol, 1eq) in toluene (10mL) under reflux. After 1h the reaction was poured into a saturated sodium bicarbonate solution (10 mL) and extracted with ethyl acetate (3 × 10 mL). The extract was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was distilled (70 °C, 33mm/Hg) to give **(*S*)-74** (1.9g, 60%) a colourless oil. Proton NMR was identical to an authentic sample.

$[\alpha]_D$ –73.1 (c. 3.3 in chloroform).

The preparation (*S*)-α-chlorophenyl acetic acid 2-chloroethyl ester (*S*)-74



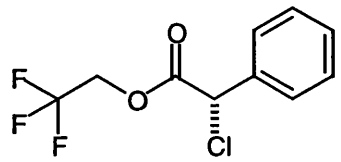
(*S*)-74

Thionyl chloride (0.17 mL, 2.3 mmol, 1eq) was added to **(*R*)-83** (0.5 g, 2.33 mmol, 1eq) in toluene (10 mL) under reflux. After 1h the reaction was poured into a saturated sodium bicarbonate solution (10 mL) and extracted with ethyl acetate (3 × 10 mL). The extract was dried with $MgSO_4$ and concentrated *in vacuo*. The residue

was distilled (120 °C, 1.5 mm/Hg) to give (*S*)-**74** (0.35g, 65%) a colourless oil. Proton NMR was identical to an authentic sample.

$[\alpha]_D$ –70.4 (c. 2.3 in chloroform).

The preparation (*S*)-α-chlorophenyl acetic acid trifluoroethyl ester (*S*)-75

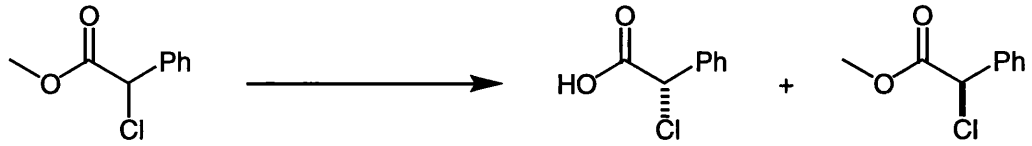


(*S*)-**75**

Thionyl chloride (0.16 mL, 2.1 mmol, 1eq) was added to (*R*)-**81** (0.5 g, 2.1 mmol, 1eq) in toluene (10 mL) under reflux. After 1h the reaction was poured into a saturated sodium bicarbonate solution (10 mL) and extracted with ethyl acetate (3 × 10 mL). The extract was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was distilled (92 °C, 1.5 mm/Hg) to give (*S*)-**75** (0.40g, 76%) a colourless oil. Proton NMR was identical to an authentic sample.

$[\alpha]_D$ –122.5 (c. 2.8 in chloroform).

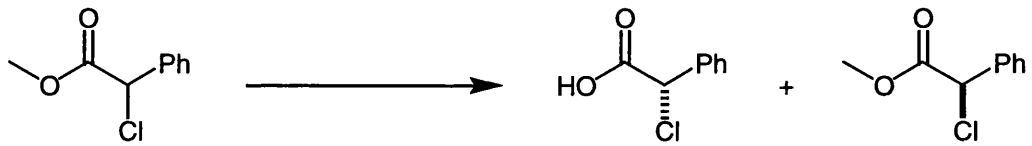
The enzymatic hydrolysis of α-chloro esters Method A



72 **(*R*)-64** **(*S*)-72**

Pseudomonas cylindracea lipase (Altus 17, CLEC) (3 mg, 10% w/w) was added to a solution of **72** (25mg, 0.14 mmol) in phosphate buffer:solvent (5 mL, 80:20) at r.t. At a suitable time the resulting solution was filtered through celite, acidified with 0.1M HCl, extracted with ethyl acetate (3 × 5 mL). The extracts dried with $MgSO_4$ and concentrated *in* vacuo, giving ester **(*S*)-72** and acid **(*R*)-64** respectfully as observed by proton NMR.

The enzymatic hydrolysis of α-chloro esters Method B

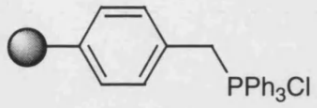


72 **(*R*)-64** **(*S*)-72**

Pseudomonas cylindracea lipase (Altus 17, CLEC) (3 mg, 10% w/w) was added to a solution of **72** (0.1 g, 0.54 mmol) in water:solvent (40 mL, 80:20) at r.t and the reactions controlled at pH 7-8 using an auto titrator. At a suitable time the resulting solution was filtered through celite, acidified with 0.1M HCl, extracted with ethyl

acetate (3 × 5 mL). The extracts dried with $MgSO_4$ and concentrated *in vacuo*, giving ester **(*S*)-72** and acid **(*R*)-64** respectfully as observed by proton NMR.

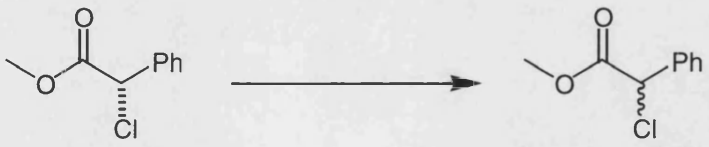
The preparation of 86



86

Triphenyl phosphine (1.0g, mmol, 10 eq.), has added to a solution of Merrifield resin (0.5g, 1.26 nM/g, 1 eq), in toluene (15 mL). The reaction was refluxed for 16h, filtered then washed successively with toluene (10 mL), water (10 mL), ethanol (10 mL) then finally dichloromethane (10 mL). The conversion of the reaction was calculated by weighing the recovered triphenyl phosphine. The resin was used immediately.

The general procedure for racemisation studies

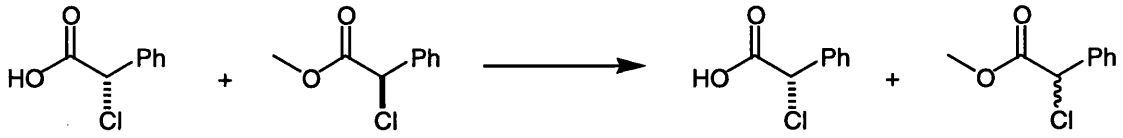


(*R*)-72 **(*R/S*)-72**

Racemising agent was added to a solution of **72** (25mg, 0.14 mmol) in phosphate buffer:solvent (5 mL, 80:20) or alternatively pH controlled water:solvent (80:20). At

a suitable time the resulting solution was filtered through silica, acidified with 0.1M HCl, extracted with ethyl acetate (3 × 5 mL). The extracts dried with $MgSO_4$ and concentrated *in vacuo*, giving ester **72** that was analysed using HPLC.

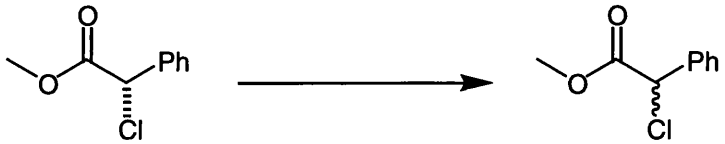
The general procedure for competition racemisation studies



(*R*)-64 (*S*)-72 (*R*)-64 (*R/S*)-72

Racemising agent was added to a solution of (*S*)-**72** (25mg, 0.14 mmol) and (*R*)-**64** (0.25 mg, 0.15 mmol) in phosphate buffer:solvent (5 mL, 80:20) or alternatively pH controlled water:solvent (80:20). At a suitable time the resulting solution was filtered through silica, the phases separated and the aqueous layer acidified with 0.1M HCl and extracted with ethyl acetate (3 × 5 mL). The extracts dried with $MgSO_4$ and concentrated *in vacuo*, giving ester (*R/S*)-**72** and (*R*)-**64** that was analysed using HPLC.

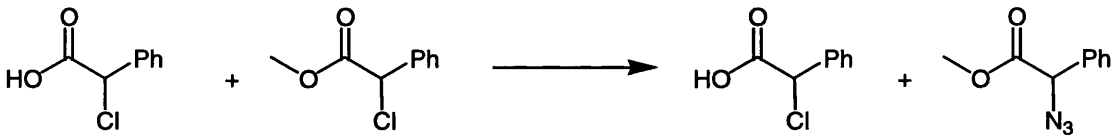
Racemisation studies in deuterated methanol – mechanism study - enolisation



(*R*)-72 (*R/S*)-72

Racemising agent was added to a solution of **72** (25mg, 0.14 mmol) in pH controlled water:MeOD (80:20) at r.t. At a suitable time the resulting solution was filtered through silica, acidified with 0.1M HCl, extracted with ethyl acetate (3 × 5 mL). The extracts dried with $MgSO_4$ and concentrated *in* vacuo, giving ester **(*R/S*)-72** that was analysed using HPLC and NMR taken to determine if any deuterium had been incoropated into **(*R/S*)-72**.

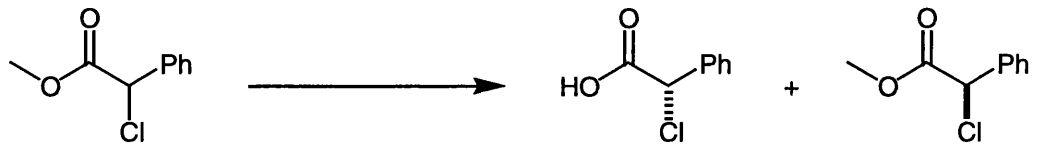
Racemisation studies in the presence of sodium azide



64 **72** **64** **87**

Sodium azide (0.020g, 0.29 mmol, 1eq) was added to a solution of **64** (25mg, 0.14 mmol) and **72** (0.25 mg, 0.15 mmol) in pH controlled water:solvent (80:20). At a suitable time the resulting solution was separated and the aqueous layer acidified with 0.1M HCl and extracted with ethyl acetate (3 × 5 mL). The extracts dried with $MgSO_4$ and concentrated *in vacuo*, giving ester **87** and acid **64** that was analysed using NMR to determine azide formation.

The *dynamic kinetic resolution* of phenyl acetic acid.



72 **(*R*)-64** **(*S*)-72**

Pseudomonas cylindracea lipase (Altus 17, CLEC) (3 mg, 10% w/w) was added to a solution of **72** (25mg, 0.14 mmol) and racemising agent (0.1-0.6 eq) in phosphate buffer:solvent (5 mL, 80:20) or pH controlled water water:solvent (5 mL, 80:20). At a suitable time the resulting solution was filtered through celite, the phases separated and the aqueous phase acidified with 0.1M HCl then, extracted with ethyl acetate (3 × 5 mL). The extracts dried with $MgSO_4$ and concentrated *in* vacuo, giving ester (***S***)-**72** and acid (***R***)-**64** respectfully, which were analysed by chiral HPLC.

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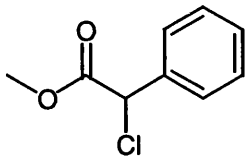
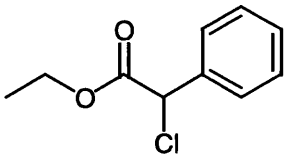
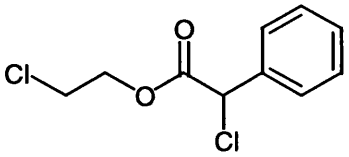
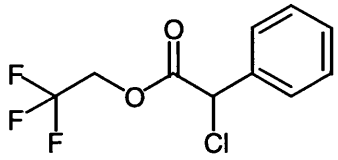
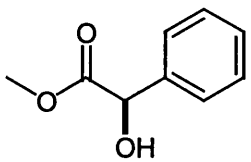
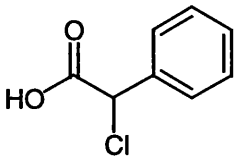
6.6 Appendix I High Performance Liquid Chromatography

Column used: Chiracel OD, 25 cm × 0.46 cm ID

	99:1 hexane: IPA, 1 ml/min 7.13 min **R** 7.90 min **S**
	99:1 hexane: IPA, 1 ml/min 7.29 min **R** 8.09 min **S**
	99:1 hexane: IPA, 1 ml/min 12.84 min **R** 16.37 min **S**
	99:1 hexane: IPA, 1 ml/min 9.36 min **R** 10.12min **S**
	99:1 hexane: IPA, 1 ml/min 13.3 min **R** 14.7 min **S**



240:10:1 hexane:IPA:formic acid, 1 ml/min

13.97 **R**

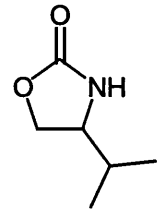
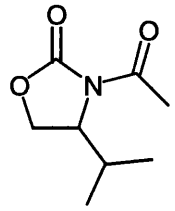
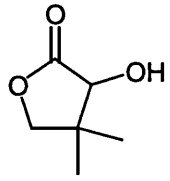
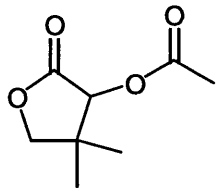
15.08 **S**

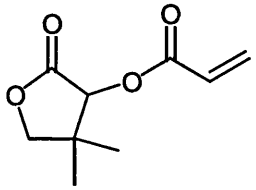
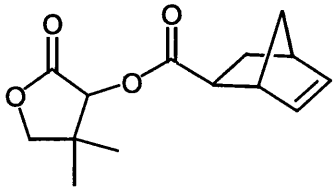
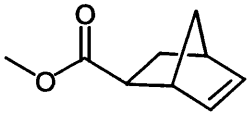
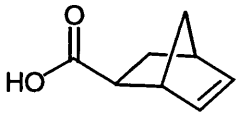
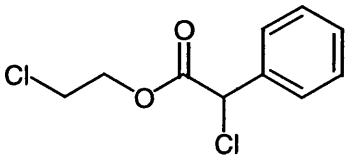
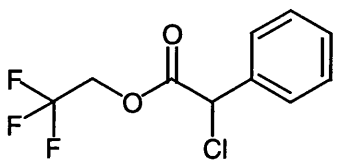
6.7 Appendix II - Gas Chromatography Separations

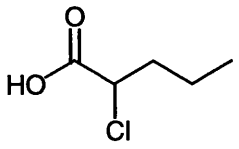
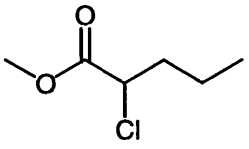
Columns used:

β-DEX-120
Fused silica capillary column
60m, 0.25 mm ID, 0.25 μm
film thickness.

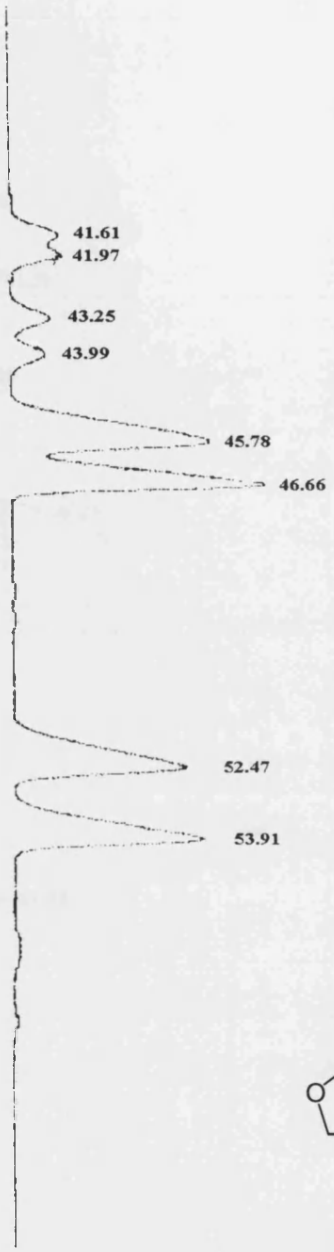
γ-DEX-120
Fused silica capillary column
30m, 0.25 mm ID, 0.25 μm
film thickness.

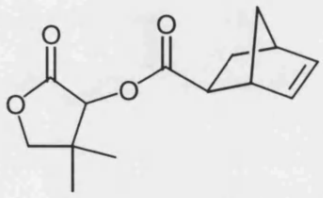
	.β-DEX, 150 °C, 16.08, 16.70 min
	β-DEX, 160 °C, 30.25, 31.31 min
	γ-DEX, 110 °C, 9.54 min **R** 10.21 min **S**
	γ-DEX, 110 °C, 16.48 min **R** 17.57 min **S**

	γ-DEX, 110 °C, 25.15 min **R** 26.45 min **S**	
	γ-DEX, 140 °C,	
	exo	*endo*
	38.74 min	43.27 min **S**
	39.08 min	43.30 min **R**
	40.26 min	48.69 min **S**
	42.49 min	50.02 min **R**
	γ-DEX, 120 °C,	
	exo	*endo*
	28.00 min	33.90 min
	28.20 min	34.70 min
	β-DEX, 170 °C,	
	exo	*endo*
	14.21 min	16.43 min
		17.02 min
	β-DEX, 150 °C, 24.36 min 24.73 min	
	β-DEX, 150 °C, 15.15 min 15.55 min	

	γ-DEX, 150 °C, 22.25 min 23.18 min.
	γ-DEX, 110 °C, 15.90 min 16.21 min.

6.8 Appendix III– GC Chromatographs





Appendix III – GC Chromatographs